PLANT PHYSIOLOGY

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TABLE OF CONTENTS

Number 1. January

Progress of plant physiological investigations in Czechoslovakia. Sylvestr Prát 1–3

Recovery processes of ponderosa pine reproduction following injury to young annual growth ..... C. K. Cooperrider 5–27

Urease distribution in *Soja max* ..... Sam Granick 29–54

Specific factors other than auxin affecting growth and root formation ..... F. W. Went 55–80

Bud regeneration and electrical polarities in *Phaseolus multiflorus* W. S. Rehm 81–101

Upward movement of inorganic solutes as affected by a girdle. Harry F. Clements and Charles J. Engard 103–122

Some absorption spectra of leaf extracts ..... G. Mackinney 123–140

Effect of light on CO_2 in leaves ..... John Shafer, Jr. 141–156

Effect of hydrogen-ion concentration on *Chlorella* photosynthesis. Robert Emerson and Lowell Green 157–168

Soil moisture as an indication of root distribution in deciduous orchards ..... F. J. Veihmeyer and A. H. Hendrickson 169–177

Effect of soil moisture on the rate of growth of longleaf and slash pine seedlings ..... L. J. Pessin 179–189

Time factor concerned with fluidity changes in warmed and cooled cells of *Zygnema* and *Spirogyra* ..... Henry T. Northen 191–198

Meta- and pyrophosphate within the agal cell. Anna L. Sommer and Thomas E. Booth 199–205

Preparation of inulin for use in adsorption columns. H. A. Spoehr 207–208

Effects of x-rays on seeds ..... A. A. Bless 209–211

Notes 213–218

Number 2. April

Preliminary experiments on the relation of growth-promoting substances to the rest period in fruit trees. J. P. Bennett and Folke Skoog 219–225

Plant diastase in evidence as to the formation and structure of starch granules ..... George L. Teller 227–240

Respiratory block in the dormant spores of *Neurospora tetrasperma* ..... David R. Goddard and Paul E. Smith 241–264

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Ammonium nutrition and metabolism of etiolated seedlings. LELAND BURKHART 265–293
Structure of the starch granule ... C. L. ALSBERG 295–330
A chemical and physiological study of traumatin, a plant wound hormone ... JAMES BONNER and JAMES ENGLISH, JR. 331–348
Effect of certain ions, used singly and in combination, on the growth and potassium, calcium, and magnesium absorption of the bean plant ... ROBERT L. CAROLUS 349–363
Chemical and spectroscopic analysis of phloem exudate and parenchyma sap from several species of plants. CARLETON A. MOOSE 365–380
Carbohydrate metabolism of *Vitis vinifera:* hemicellulose. A. J. WINKLER and W. O. WILLIAMS 381–390
Accessory salts in the nutrition of excised tomato roots. PHILIP R. WHITE 391–398
Influence of foliage sprays on drought resistance of conifers. HARDY L. SHIRLEY and LLOYD J. MEULI 399–406
Formation of nitrate in detached green leaves of Swiss chard and tomato ... MARY C. MCKEE and DOROTHY E. LOBB 407–412
Formation of carotenoids and chlorophylls in etiolated barley seedlings exposed to red light ... HAROLD H. STRAIN 413–418
A photoelectric photometer for chemical analysis. B. N. SINGH and N. K. ANANTHA RAO 419–425
Applicability of KUNDT'S rule to chlorophyll ... G. MACKINNEY 427–430
Notes ... 431–436

NUMBER 3. JULY

Hormones and the analysis of growth ... KENNETH V. THIMANN 437–449
Exanthema in pear, and copper deficiency. JACOB OSERKOWSKY and HAROLD E. THOMAS 451–467
Water relations in *Bryophyllum calycinum* subjected to severe drying ... WALTER BURCHARD WELCH 469–487
Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and its effects on nitrogenous and carbohydrate constituents. C. P. SIDERIS, B. H. KRAUSS, and H. Y. YOUNG 489–527
Electrical polarity and auxin transport ... W. G. CLARK 529–552
Conductivity measurements of plant sap ... GLENN A. GREATHOUSE 553–569
Behavior of pectic substances and naringin in grapefruit in the field and in storage ... G. L. RYGG and E. M. HARVEY 571–586
Auxin production in seedlings of dwarf maize. J. VAN OVERBEEK 587–598

Correlation between growth of excised root tips and types of food stored in the seed ..... GLADYS C. GALLIGAR 599–609
Intermediates of vitamin B_1 and the growth of *Torula*.
WILLIAM J. ROBBINS and FREDERICK KAVANAGH 611–619
Relationship of the organic acids of tobacco to the inorganic basic constituents.
GEORGE W. PUCHER, HUBERT BRADFORD VICKERY, and ALFRED J. WAKEMAN 621–630
Growth of cabbage seedlings in sand culture, as affected by delayed application of nutrient salts ..... A. A. DUNLAP 631–640
Isolation of a hexosemonophosphate from pea leaves ..... W. Z. HASSID 641–647
Influence of osmotic pressure on sporulation by *Bacillus subtilis*.
JAMES L. ROBERTS, WELDON C. WHITE, and ELIZABETH OJERHOLM 649–653
Influence of the length of day on the dormancy of tree seedlings.
FELIX G. GUSTAFSON 655–658
Effect of drought on protoplasmic elasticity ..... HENRY T. NORTHEN 658–660
Notes 661–668

NUMBER 4. OCTOBER

Diurnal fluctuation in root pressure ..... KARL A. GROSSENBACHER 669–676
Foliar diagnosis: its relation to the optimum nutrition of the potato ..... WALTER THOMAS 677–694
Effects of a limiting element on the absorption of individual elements and on the anion: cation balance in wheat.
A. G. MCCALLA and E. K. WOODFORD 695–712
Relation between light and the electric polarity of *Chara*.
SIDNEY O. BROWN 713–736
A study of the mineral nutrition of wheat as influenced by fertilizer combinations ..... FRANK T. DONALDSON 737–766
Salt tolerance of plants at different temperatures.
S. M. AHI and W. L. POWERS 767–789
Translocation in plants ..... A. S. CRAFTS 791–814
Ice formation and the death of plant cells by freezing.
IRENE H. STUCKEY and OTIS F. CURTIS 815–833
Temperature effects upon the growth of excised root tips.
GLADYS C. GALLIGAR 835–844
Carbohydrates of bean plants after treatment with indole-3-acetic acid ..... TAYLOR R. ALEXANDER 845–858
The MIRSKY-PAULING theory of the structure of native, denatured, and coagulated proteins, and some theoretical aspects of the evolution of oxygen from the irradiated green plant.
O. L. INMAN 859–862

Heritable variations in chlorophyll ..... J. C. IRELAND 863–865
Nicotinic acid and the growth of isolated pea embryos. JAMES BONNER 865–868
Effect of flue dust on the growth of sunflower and Golden Wax bean ..... BARNEY BARNETT COHEN 868–871
Effect of sun and shade on pigment development ..... W. A. BECK 871–872
Teaching methods in plant physiology ..... CHARLES J. LYON 873–875
Notes 877–882
General Index 883–893

ERRATA

VOLUME 13

Page 121, citation no. 1, for "Asrbury" read Astbury.
Page 126, figure 2, arrange wave length designations as in figure 1, page 125.
Page 127, figure 3, arrange wave length designations as in figure 1, page 125.
Page 128, figure 4, arrange wave length designations as in figure 1, page 125.
Page 243, and odd numbered pages to page 263, running head, for "INDORMANT" read IN DORMANT.
Page 384, line 2, delete "however."
Page 384, last line, insert "otherwise" after "have."
Page 386, line 4 from bottom, insert "direct" before "correlation."
Page 386, line 3 from bottom, omit dashes and read "cell walls and structural rigidity."
Page 388, delete citation no. 15.
Page 390, delete citation no. 35.

BOHUMIL NĚMEC
MARCH 12, 1873

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PLANT PHYSIOLOGY

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PROGRESS OF PLANT PHYSIOLOGICAL INVESTIGATIONS IN CZECHOSLOVAKIA

SILVESTR PRÁT

(WITH ONE PLATE)

The country Bohemia (Čechy) in Czechslovakia (Československo), and especially the Czechoslovak capital Praha Prague, has a prominent position in the history of plant physiology. Five years ago the hundredth anniversary of the birthday of JULIUS SACHS as founder of plant physiology was commemorated (PLANT PHYSIOL. 7: 179, 750. 1932). It is necessary to emphasize that SACHS started his investigations in Praha. He was called to Praha by J. E. PURKYNĚ, professor of physiology at the medical faculty. On December 17, 1937, we celebrated the 150th anniversary of the birth of physiologist PURKYNĚ. JAN EV. PURKYNĚ was not only inventor of microscopic technique but he also was the author of the cellular theory; he did not publish anything special in plant physiology, unless we consider the book *De cellulis antherarum fibrosis* as a thema belonging to physiological anatomy. But there is no doubt that PURKYNĚ was a teacher and leader of his young collaborator, J. SACHS. In the laboratory of PURKYNĚ in Praha, SACHS learned the experimental methods; in Praha he finished his studies, got his doctor degree (1856), and was the first privat docent for plant physiology in the whole world (1857). About twenty papers by SACHS are published in the Czech journal *Živa*. The river Vltava in Praha furnished the water for the water-cultures in SACHS's experiments, and the success of this work was the cause of SACHS being called, in the year 1859, to Tharandt in Germany. For a long time after SACHS left Praha, there was practically no development of plant physiology in Czech countries. But we must mention, that in the year 1866, G. MENDEL published in Brno (Moravia) his experiments about cultures of plants which later on were the basis of genetics.

In the year 1872, a plant physiological laboratory was established at the German university of Praha for Prof. A. WEISS. His successors were H.

MOLISCH, the developer of plant microchemistry, and F. CZAPEK, known for his *Biochemie der Pflanzen.*

In the Czech university of Praha, known as Charles University, not till in the year 1898 was a laboratory for plant physiology founded, and opened in 1900 under the direction of Dr. BOHUMIL NĚMEC. His laboratory was at the beginning very small and very poor; but soon it became very well known, not only in his country but also in foreign countries. Many biologists from all countries are drawn to his laboratory by the attractive personality of Professor NĚMEC. He brought plant physiology into close contact with cytology. NĚMEC obtained his first training in scientific work in the zoological laboratory of Professor Dr. F. VEJDOVSKÝ; and his first publications are in zoology. The knowledge of zoology often proved invaluable in the work of Prof. NĚMEC both in general conceptions, as for instance in the application of zoological microtechnic in botany; and in details, as, *e.g.*, in the study of zoocecidia in plants and their relation to physiological anatomy. As early as 1899, it was shown clearly by NĚMEC that there is a wave in the viscosity of protoplasm during mitosis, a fact which, nearly forgotten, was not until thirty years later rediscovered, and then led to a detailed analysis of the cell from the viewpoint of colloid chemistry. NĚMEC was the first investigator to publish measurements of the viscosity of protoplasm (1901) in relation to temperature (measured by the speed of fall of starch grains); and his especially keen biological intuition is shown in the fact that he used intact plants or tissues, and not only sections, in his experiments. These facts lead to the conclusion that the basis of colloid chemistry of living matter and of experimental cytology was laid down in Czechoslovakia about the year 1900 by NĚMEC. He also devoted much time and effort to the problem of the structure of the cell, nucleus, and chromosomes (the first photomicrographs of living chromosomes in ultraviolet radiation were made in collaboration with Prof. KRUIS). The theory of statolith starch grains in geotropic responses, first defined by NĚMEC, was cited and accepted by HABERLANDT, and now as generally known and acknowledged, must be considered in any thorough discussion of geotropism.

Extensive study concerning regeneration is a theme to which NĚMEC at different periods of his work has returned. Not only the regeneration of roots and leaves, but special cases of regeneration in fungi must be mentioned here. In the hard tissue of wood fungi (Polyporaceae) which never before were used in physiological experiments, very good objects of astonishing regenerative capacity and reactivity were found by NĚMEC. The regeneration studies are closely related to experimental morphology in which NĚMEC published some papers concerning development, dorsiventrality, and other problems which are handled not only descriptively but are always discussed and worked through in close relation to physiology.

NĚMEC made many studies on irritability, conductibility, reactivity, and tropisms of plants. In extensive studies on mixoploidy NĚMEC is studying the problem of reactivity of the organism and the principal problems of individuality. The last epoch of work of NĚMEC includes extensive studies about minor elements in plants, based on exact analysis of ash; the experiments are conducted with many collaborators.

The Czech students are obliged to Professor NĚMEC for excellent text books in plant anatomy, physiology, and general biology. Notwithstanding the fact that they are written in the Czech language, their illustrations are praised and highly esteemed in foreign countries.

The bibliography of the works by Prof. NĚMEC is too long to be reprinted here; it is *in extenso* published in *Preslia* **2**: 5–12. 1923, and supplemented in *Preslia* **11**: 3–10. 1932; **12**: 69–70, 117–118, 164–166, 207. 1934; **13–15**: 337–338. 1936.

Professor Dr. B. NĚMEC, the first professor of anatomy and physiology of plants at Charles University, can be said to have brought plant science in Czechoslovakia to the peak of its development. He is conducting not only his own scientific work, but he is also incorporating his work and that of his many students into the international fabric of botany. His work has exerted a profound influence upon the development of botanical science during the last forty years, and will continue undoubtedly for many decades as one of the major forces in determining trends of botanical research. All research and experimentation developed rapidly in Czechoslovakia after the World War. The foundation of new universities, schools, and research institutions made possible a great extension of the work. Nearly all botanists working and teaching in Czechoslovakia, and some in foreign countries, are students educated in the laboratory of Professor NĚMEC; and the progress of Czechoslovak plant-sciences can be followed in publications not only in the Czech but also in international scientific journals.

CHARLES UNIVERSITY
PRAHA, ČESKOSLOVENSKO

RECOVERY PROCESSES OF PONDEROSA PINE REPRODUCTION FOLLOWING INJURY TO YOUNG ANNUAL GROWTH[1]

C. K. COOPERRIDER

(WITH THIRTEEN FIGURES)

Introduction

In the plateau region of northern Arizona peculiar geologic formations account for the lack of the living natural waters that are typical of most high mountain districts. They also exert a profound influence on the plant growth, and the character of forage through their effect on soil and available moisture. In the forested areas most affected by these formations animals are particularly apt to browse pine reproduction. Tip moths are also very destructive in places, sometimes over large areas. Thus control of injury to young trees, the future forest, is an important problem in the management of these forest lands.

In order to exercise proper control, it is necessary to know at least something of what constitutes serious injury. This depends not only on the degree to which pine seedlings are damaged but also on the power they have to recover. This recovery phase of the problem was investigated in connection with a long-time range-timber reproduction study. Recovery was found to depend principally on the powers ponderosa pine has to replace lost shoots through the development of extraordinary buds.

Literature

A review of the literature has revealed but little concerning the power of pines to replace those shoots browsed off by animals. BÜSGEN and MÜNCH (**1**) say, "In general the tendency to epicormic branch formation is less in conifers than in broad-leaved trees. Our Scots pine opens dormant buds only in extremity, after complete defoliation by caterpillars. A few dwarf shoots with ordinary double needles may then be formed from dormant whorl buds, and the dormant eyes between the destroyed needles of the dwarf shoots may become rosette shoots. . . ."

BÜSGEN and MÜNCH continue their discussion with statements concerning conifers, as follows: "The North American *Pinus rigida*, not infrequently cultivated in Germany . . ., develops preventitious shoots very readily even on old stems." (No statement pertaining to the circumstances of development is made.) These authors state further that "the clothing of the stem with green and the replacement of lost shoots,

[1] Publication of the Senior Range Examiner at the Southwestern Forest and Range Experiment Station, maintained at Tucson, Arizona, by the Forest Service, United States Department of Agriculture, in cooperation with the University of Arizona.

besides being brought about by dormant buds produced normally on the young annual shoots (preventitious buds) may be effected through so-called secondary buds . . . in the axils of leaves or bud scales in which no perfected bud would normally have developed. The power of producing such secondary buds is possessed especially by the spruce, and on this to a great extent depends the inexhaustible power of recovery after cutting or grazing which makes it so suitable a hedge plant."

This "inexhaustible" power of spruce trees to recover was observed years ago on Engelmann spruce (*Picea engelmanni*) on too frequently used sheep bed grounds in the high mountains of northern New Mexico.

When substitute buds were observed on ponderosa pine (1925), it was considered most unusual, for there seemed to be a common belief that loss of terminal buds or shoots cut short the development of young pines and that when the primary leader and lateral were destroyed, recovery depended on the presence of an uninjured lower branch. Before this, in writing of sheep injuries to ponderosa pine in Idaho, SPARHAWK (**5**) recognized recovery through the ascendancy of a side branch and probably the development of buds also when he said, "An uninjured side branch or a new bud takes the place of the leader. . . ." HILL (**3**) also recognized the common occurrence of recovery where the leading growth was destroyed but does not describe any recovery process. During recent years several authors, including GRAHAM and BAUMHOFER (**2**), KIENHOLZ (**4**), and WAKELEY (**6**), have described growth from substitute buds following tip moth and frost injury to pines.

Observations

Unmistakable evidences of substitute bud and shoot development on browsed ponderosa pine seedlings were observed during the late summer of 1925 and again in 1926 while working range study plots in the forests of northern Arizona. In 1927 intensive investigations on the relation of grazing to natural regeneration on cut-over timberlands were initiated in the same district. These studies, which were continued over a period of 10 years, offered the opportunity to determine the processes and circumstances of extraordinary shoot development. In addition, observations were extended to the main pine forest areas of Arizona and New Mexico and to some extent to those of southern Colorado.

Cattle and browsing game animals, as well as sheep and tip moths, did the principal amount of injury to seedlings older than 3 years, whereas cutting off top parts of the younger seedlings was done mainly by rodents. Browsing by livestock and game animals, although common and severe in some districts, was lacking or almost so in other districts.

The replacement of lost current growths through the development of substitute buds or shoots proved to be common. It was also found that

there are several different processes whereby ponderosa pine develops substitute buds and shoots, particular development depending on a number of circumstances or factors, such as time when injury occurred in relation to annual shoot growth, factor causing injury, and the parts of a plant removed or injured.

Processes of recovery

Substitute growths

The replacement of lost shoots on older plants takes place on stubs of shoots left and on the stems from which shoots have been removed.

Substitute growths on young shoots.—These may open when needles are left on the stubs of injured shoots through development of (a) needle-bundle buds between the needles of the uppermost bundles left, and (b) rarely through whorl buds and the secondary buds in the axils of scales; but stubs without needles die.

Substitute growths on old stems.—Such growths may develop from (a) dormant whorl buds, (b) secondary buds in the axils of scales from which no short shoot sprang, and (c) buds of unknown origin herein discussed under *other substitute growths.*

Substitute growths in juvenile seedlings.—In plants of the cotyledon stage these growths commonly arise from (a) dormant buds that are a part of the growth point above the cotyledons, and in 1- and 2-year-old plants from (b) dormant buds in the axils of primary leaves. None of these different growths are considered adventitious, because they arise from buds formed in due order or from the dormant eye between the needles of short shoots. This excellent statement concerning adventitious buds is taken from Büsgen and Münch: "They must not be confused with the dormant buds . . ., which while they may indeed produce shoots out of proper order, were nevertheless originally formed in due order from cells whose active formation has already ceased. This is the case, for example, with *Begonia* leaves which the gardener cuts off and places in moist sand for propagation purposes."

In order to make clear the different processes of substitute shoot development, the normal growth habits of pines will be briefly presented here.

Pines 3 years and older have two distinct forms of branches or shoots, long and short. Long shoots bear the scale leaves and short shoots (also known as modified stems, dwarf shoots, and spurs) the needle bundles or foliage leaves. There is an entirely different behavior in young pines less than 3 years old. On the first growth above the smooth cotyledons and also on the leading growth of the second year are borne spirally arranged single-toothed leaves known as primary leaves, so closely spaced as to

resemble tufts. Branching begins the third year with the development of long shoots.

SUBSTITUTE GROWTHS ON YOUNG ANNUAL SHOOTS.—Unstimulated dormant buds (whorl buds and secondary buds) and likewise the eye initial within each needle bundle remain asleep. Either may awaken when the buds or growth point above them are destroyed. The inconspicuous dormant whorl buds lie close below the shoot's tip, hence they seldom escape being taken with the part of the shoot browsed off by animals. Likewise, if tip moth larvae kill a shoot's tip, the dormant whorl buds are destroyed also. Secondary buds (dormant buds in the axils of scales and from which no short shoots grew) develop only when foliage persists to keep the stub alive, and then only rarely. Needle bundles, on the other hand, are usually left on the stubs of browsed shoots and development of eye initials is common.

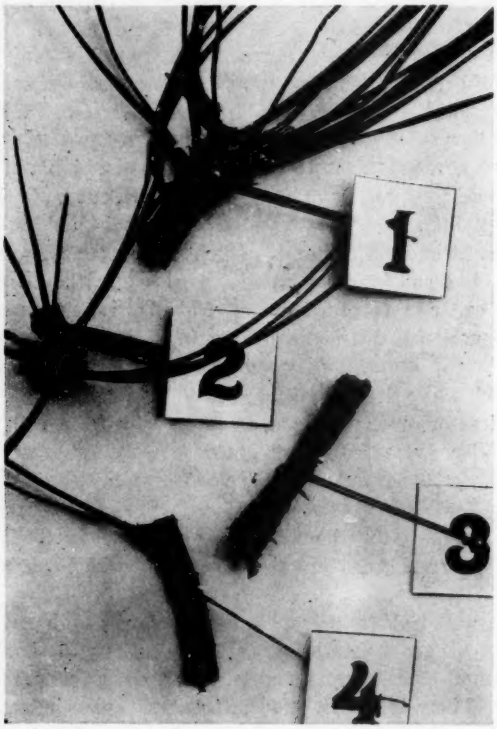

FIG. 1. Stubs of browsed shoots: 1, resin-capped stub with several needle bundles, showing early stages of bud development in the two uppermost bundles; 2, stub with a very young bud (from a small plant); 3, stub (dying for lack of foliage); 4, stub with only one needle bundle in which bud development has begun.

NEEDLE-BUNDLE BUDS

Browsing animals and tip moths attack young shoots in early summer, mainly during the latter two-thirds of the period of elongation. When browsing, animals jerk off growing terminals, usually leaving stubs 1 or more inches in length with needle bundles. Development of the eye initial in one or more of these soon begins. A similar development takes place following tip moth injury, but the effects of tip moth continue throughout the several weeks' period of development of the larvae in a shoot and subsequent bud development is much less likely to take place. Stubs without needle bundles soon wither and die.

Typical development of needle-bundle buds takes place on stubs of browsed-off young shoots, such as those in figure 1. The stub of a browsed-off shoot exudes a clear sticky resin that soon hardens and covers the injured tissues. The earliest indication of bud development is a slight swelling

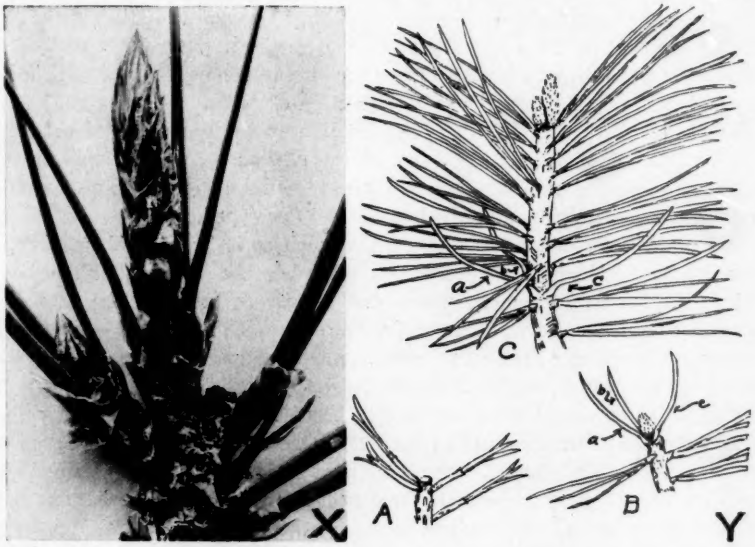

FIG. 2. X. Needle-bundle buds on the stub of a shoot that was browsed in June (photographed in August). The bud on the left is typical of development in the fall, following injury. The bud on the right has begun growth. Some needles were removed to aid photographing. Y. Three stages in the development of an extraordinary bud or shoot from an eye initial. A, stub of a shoot soon after injury, showing the usual stage of needle growth when browsing begins. B, the same stub, in August, with a well-developed needle-bundle bud. C, extraordinary shoot (on stub shown in B) 1 year later. Note the peculiar needles (*a*, *b*, *c*) of the bundle from which the substitute growth opened. Note also how perfectly the new shoot has become a part of the stem.

within the paper-like sheath of one or more of the uppermost needle bundles on a stub. Such development becomes noticeable about 30 or 40 days after injury, but a slightly magnified section of the uppermost needle bundle, the one most likely to develop a bud, will show development of the eye initial in a much shorter time. Buds that appear early in the summer develop rapidly and may elongate into shoots several inches long by October. Current development usually is confined to buds (never more than one to a short shoot), hereinafter called needle-bundle buds which, in the fall, resemble the terminal buds of normal shoots (fig. 2).

During the period of development, such a bud swells, breaks the needle sheath, and pushes the needles apart until they appear to be single leaves on a short shoot which now bears a well-developed bud. Thus such a short shoot has begun to function as a long shoot.

The part of any grazed-off stub above its uppermost needle bundle soon withers and sloughs away. As the young bud develops, it turns upward and, particularly on the stub of a primary shoot, gradually pushes the sloughing tissues above it away and becomes the new terminal bud of the injured shoot (B in Y, fig. 2). Recovery through the growth of such a bud often is so complete that the only indication of past injury is a rind union or crease which resembles a finger-joint line on the palm side of the hand. This crease is formed by the union of the new shoot, which turns upward across the injured end of the stem, with the rind on the opposite side of the old stem from which the needle-bundle bud developed.

Change in function of a short shoot, such as is brought about through development of its eye initial, also is indicated by the needles the shoot bears. These needles attain abnormal size and become so broad at the base as to resemble the leaves of the century plant or agave (X, fig. 2). They set early, whereas normal needles remain soft at the base and may be easily pulled from the sheath so long as they grow, usually until late fall. The needles surrounding a needle-bundle bud also become so fibrous and firmly fixed that they are never shed as are the normal needles but, unless knocked off, persist even after death. Some plants kept under observation retained such needles after 9 years. In fact, these peculiar needles, which become fixed because the short shoot on which they grow becomes a stem and is not shed with the leaves as ordinary short shoots are, furnish unmistakable evidence of former injury. They have been found persisting on the lower part of the main stems of trees, as the one in A of figure 3, that were from 5 to 25 feet tall.

Incidentally, the needles of bundles from which buds spring sometime clearly show that the foliage leaves, which may be short when shoots are browsed, grow from the base and not from the tip end. The needles are particularly apt to adhere to the sheath of a bundle in which bud develop-

FIG. 3. Main stem of a ponderosa pine 5 years after injury from browsing. Note how straight it is. A, single old needle (one of the original bundle shown in B), and, immediately above it, a line where the rind of stub and substitute shoot joined. B, needle-bundle bud through which recovery took place.

ment is taking place. As these needles elongate, they bow outward, being unable to extend in a straight line, burst through the sides of the sheath, and form into loops.

DORMANT WHORL BUDS AND SECONDARY BUDS ON YOUNG ANNUAL SHOOTS

Some normal shoots develop well-defined terminal buds early in summer. Injury to such leading buds without destruction of dormant whorl buds seldom occurs. Only a very few shoots have been observed on which the terminal buds had been destroyed by some undetermined factor and substitute buds formed, apparently from dormant whorl buds. In order to test for such a response, the leading terminal and lateral buds were pinched from forward shoots in midsummer. By fall such destroyed buds were replaced by one or more small but otherwise apparently normal buds which opened normal shoots the following year.

A few growths which were considered to be from secondary buds, because of their location in the axils of scales, were observed. They occur only on shoot stubs having foliage, for stubs without foliage die before new growth develops. On stubs with foliage, needle-bundle buds usually develop so readily that secondary buds remain dormant.

Development of dormant whorl and secondary buds on shoots is too rare to be of any practical importance and is described merely to show that such responses do take place.

Growths on old stems

Substitute growths.—Old stems as well as current growths may develop substitute shoots. Furthermore, when vigorous plants are pruned severely for several years, the stubs of current shoots with foliage leaves may develop needle-bundle buds and old stems bearing these stubs may develop substitute growth from whorl or secondary buds. Usually shoot development on old stems takes place in extremity, or where no stubs of shoots with needle bundles are left.

When tip moth and browsing animals severely damage young pines, leading terminal and lateral shoots sometimes are killed or pulled off close

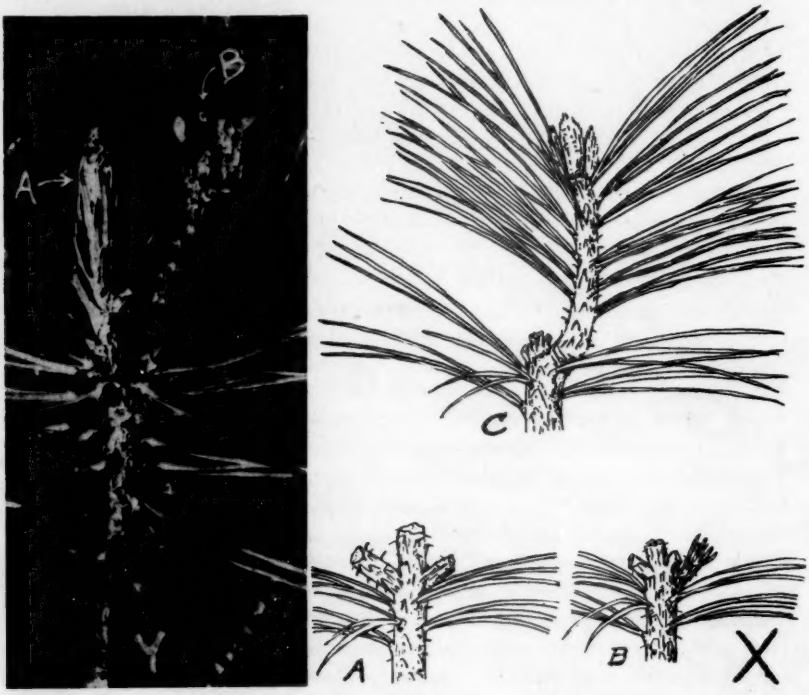

Fig. 4. X. Three stages in the development of substitute growth from a dormant whorl bud. A, main stem with three stubs of browsed shoots without any needle bundles (June). B, new shoot (a) opening from a whorl bud on the old stem (Aug.). The dead stubs have shrunk. C, substitute shoot 1 year later (Aug.). The second year following injury, the main stem was almost straight. Y. Two kinds of substitute growths on a browsed plant. A, new shoot opening from a whorl bud on old stem. B, needle-bundle bud on stub of browsed-off shoot. Note the short growths that resemble primary leaves on shoot A and the needle bundles in the axil of the lower of these leaves. The old stem is that part with mature foliage.

to the old stem bearing them. These lost shoots usually are replaced through development of either dormant whorl or secondary buds on the old stem.

DORMANT WHORL BUDS.—Whorl buds open readily from uninjured year-old stems, but where injuries occur year after year stems that have been tipped when they were shoots have no whorl buds. The absence of whorl buds on some stem growths, however, does not prevent recovery, for these buds may develop and open shoots on stems 2 or more years old.

When the growth of the old stem that bears the stubs of lost shoots (A in X, fig. 4) was not injured the previous year, the year it was a terminal shoot, one or more of its sleeping whorl buds located just below the dying stubs is almost certain to awaken. Sometimes both needle-bundle and whorl buds develop, as is the case in Y of figure 4. These whorl bud growths from their beginning resemble elongating shoots. No conspicuous buds form, as in the case of the development of needle-bundle buds. Instead, a cluster of scales and of needle points which otherwise resemble the cone of an elongating normal shoot burst forth, making shoots 1 or more inches long and sometimes much longer the year the plant was injured (B in X and A in Y, fig. 4). These current growths from whorl buds are easily distinguished by their short needles which are crowded on short stem growths, resembling the well-known secondary growth of a normal shoot, which growth also takes place so late in the season that it is stunted by low fall temperature.

Plants that have been injured and have recovered through the growth of dormant whorl buds show that such injuries may be detected with reasonable certainty years after they occur. Whorl bud growths having much longer base than top needles are cone-shaped. Any growth length of the main stem of a plant, which is much shorter than the lengths above and below it and which bears the short crowded needles referred to or their scars, is from a whorled bud, or is normal secondary growth. Such a growth, particularly one without side branches near its base (laterals are uncommon in the case of whorl bud growths), when accompanied by stubs of shoots or their scars and/or a slight crook in the main stem, is unmistakable evidence of shoot injury and recovery through bud development (fig. 5).

Where main stems are the product of uninjured shoots and stubs of shoots, dormant whorl buds may be lacking on stem growths 1 or more years old, because they have been destroyed. In such cases new shoots have been observed on stems 2 or more years old. Wood sections and the location near the tips of annual growths indicated whorl-bud origin of these shoots. Furthermore some records were taken in sufficient detail to determine which stem growths were uninjured when they were current shoots, and thus which ones might have dormant whorl buds.

SECONDARY BUDS.—Still other but rather rare-occurring substitute shoots were observed on year-old stems. They occurred on stubs of stems that had

FIG. 5. A many-times injured plant, height 18 inches, illustrating the peculiar appearance of current growth from whorl buds, accelerated growth following release from injury, and recovery. A, shoot growth (1933) from one-half inch stub (*a*) of a browsed (1932) shoot. B, late season whorl-bud growth (1931) following early summer loss of shoots. The visible part of the main stem is the product of two uninjured growths (A and B, both from extraordinary buds) and three short stubs of shoots. The uninjured 1933 growth (A) represents one-half the total height of the 13-year-old tree.

been tipped when they were shoots and from which needle-bundle-bud shoots had opened and been destroyed the following year. Thus such a year-old stem had no whorl buds left and the shoot stub it bore had neither whorl buds nor needle bundles from which substitute growth could develop. These old-stem shoots were considered to arise from dormant secondary buds because of their location with respect to the axils of scales from which no short shoots had developed. To definitely establish the fact, more detailed morphological study than has been made is necessary.

OTHER SUBSTITUTE GROWTHS

Small shoots which resemble the slow-growing terminals of oldest side branches appear from time to time on the main stems of young pines below

their oldest side branches. They open on uninjured as well as injured plants, but occur principally before the stem is covered with thick flakes of corky bark. They may last no longer than the foliage leaves they bear or they may put on puny terminal growth for only a year or two. But if the leading growths above are lost, passive main-stem shoots may become active, may put on annual shoots similar to those of normal leading growths, and may become the main stems.

Vigorous shoots similar to those of normal leading growths may also spring from the lower main stems of repeatedly damaged plants. The origin of many of these main-stem shoots seems to bear relation to the location of dormant buds. Such awakened buds push through places where the rind is thin, as scale scars and where the inner bark is exposed in vertical cracks which are so common in the smooth rind of the main stem of young pines. Sometimes vigorous shoots springing from cracks in the rind seem to bear no relation to the probable location of any dormant buds; possibly they are adventitious.

Adventitious budding is also indicated by the opening of shoots from wounds. To illustrate: A small pine was found cut off close to the ground, presumably by a porcupine. The following summer a vigorous shoot appeared above the grass at the mapped location of the cut-off plant. It sprang from the cut surface of the stump's rind but may have grown from a dormant bud. Were this the case, the rarity of their occurrences could be explained by the small number of young pines that are cut off when a stimulus for growth is on and by the poor chance that many of them would be so severed as to expose some dormant bud that still retained its vital connections with the stem.

SUBSTITUTE GROWTH IN JUVENILE SEEDLINGS.—Extraordinary budding readily takes place in seedlings in the primary leaf stage. The only animals that appreciably injured seedlings in the cotyledon stage or during the year of germination were rodents, although mortality from other causes, such as drought, was very high.

Rodents, principally ground squirrels and mice, may be very destructive. They cut off the stems of many young plants and all or a part of the cotyledons of others. Most of the injured cotyledons are taken before or while unfolding and in a manner that could not be done by any large-mouthed animal. Small rodents do this when they take the seed coats from germinating plants. This accounts for the fact that any number of the leaflike cotyledons (fig. 6A), from all to only a few, or any amount of all or only a few cotyledons may be cut off, the parts taken depending on the parts still in the seed coat when it is taken.

Obviously when a seedling was cut off below the cotyledons it died, but many rodent-damaged plants with only a few cotyledons or parts of them

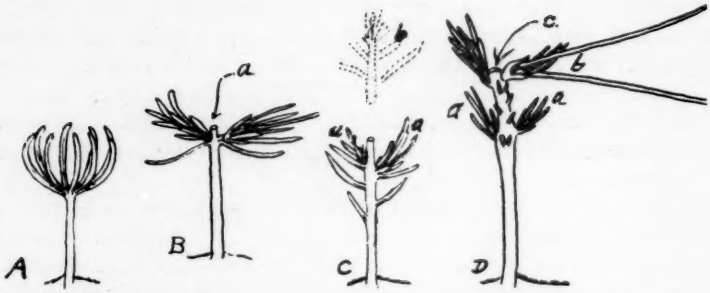

FIG. 6. Growths on young seedlings. A, cotyledon stage (ordinary growth soon after shedding of seed coat). B, substitute growths that are similar to the lost first leading growth. (The first leading growth was bitten off below all leaves (*a*). The substitute growths, with primary leaves, opened from a point above the cotyledons, which persisted.) C, substitute growths (*a*) in the axils of primary leaves. (A part of the leading growth (*b*) of the second year was lost.) D, substitute growths on a three-year-old seedling; *a*, growths, with primary leaves in the axils of primary leaves; *b*, a needle-bundle bud. (The plant's first long shoot was bitten off leaving one needle bundle on the stub (*c*) of that growth and primary leaves on the older growth.)

survived. Where such injuries occur without damage to the plumule, the plant develops normally. The first leading growth above the cotyledons also was commonly damaged by rodents and sometimes by browsing animals. Recovery from such injury took place through the opening of substitute growth at a point above the cotyledons, as is shown in B of figure 6.

GROWTH THE SECOND YEAR

Under average conditions, substantial primary growth begins when seedlings are nearly 1 year old. The leading growth above the cotyledons is so nearly covered by primary leaves that even if such growth were cut off by rodents or jerked off by browsing animals, some undamaged leaves usually were left. Buds and then new growths (one bud to a leaf) may open in the axil of one or more of the uppermost of these leaves soon after injury. They open rapidly, forming new growths (C, fig. 6) which also bear primary leaves.

Sometimes when substitute growth is injured soon after it is made, a second growth forms on the remnant of the first and after the same manner as the first. This exceptional ability to produce more than one substitute growth in a season is accounted for by the tendency of pines in the primary-leaf stage to grow throughout the summer rather than to complete growth elongation during early summer, as older plants usually do.

Growth the third year

During the third year normal seedlings form long shoots from buds nesting in the axil of some primary leaves of the second year's growth. The first long shoots may bear more than one short shoot or may be so small as to have only one with good foliage leaves. As a rule, only shoots of appreciable length are damaged by browsing animals, but tip moths injure smallest shoots also.

When a first long shoot is killed, an undeveloped bud in the axil of some primary leaf opens and a new shoot soon takes the place of the one destroyed. Should the substitute shoot be injured so as to leave a short shoot with foliage leaves, a needle-bundle bud develops after the manner already described, which is shown in D of figure 6.

Substitute budding, to be expected

It seems appropriate at this point to call attention to the relation of extraordinary buds and shoots to the normal life of pines.

As has been pointed out, young pines, beginning with the cotyledon stage, have dormant buds which possess powers of growth similar to those of primary buds.

The needle-bundle bud may be considered the most unusual of the substitute growths, unless possibly pines do produce adventitious buds, and this

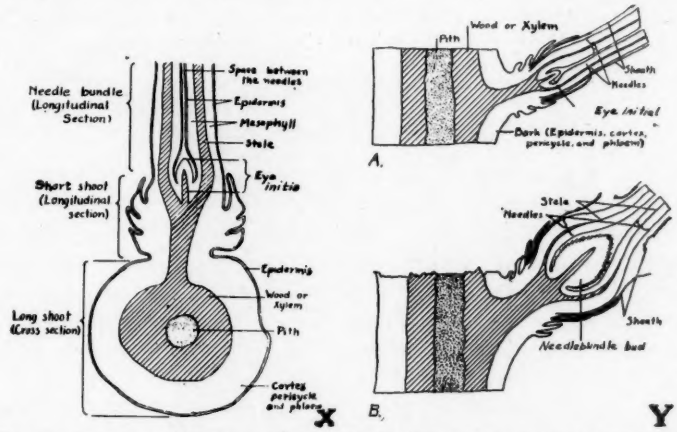

FIG. 7. X, cross-section of a young shoot and longitudinal section of one of its short shoots (ponderosa pine), showing the relation between the eye initial in the needle bundle and the shoot (enlarged 9 diameters). Y, the eye initial a growth point. A, longitudinal section (ponderosa pine) through uninjured long and short shoots, showing the connection between the eye initial and the vital parts of a young shoot. B, the stub of a browsed shoot, showing early stages in the development of the eye initial into a needle-bundle bud. (Enlarged 9 diameters.)

has not been definitely established. But are there any good reasons why development of the needle-bundle bud should be unexpected? A short shoot is but a branch of a long shoot. It retains all its connections with the parent long shoot when current growth and occasionally when 1 or 2 years old. It has an eye initial on its tip. Thus a short shoot has all the parts required for transformation into a long shoot if called on to so function (fig. 7).

At the time these observations were begun, beliefs regarding substitute budding may have been influenced by the behavior of particular pines. Perhaps the tendencies to substitute shoot development may be much less in some species than in others, or some may not form such shoots at all. GRAHAM and BAUMHOFER (2) show that tip-mothed ponderosa pine developed needle-bundle buds less readily than other pines studied at Halsey, Nebraska. On the other hand, men who were shown examples of substitute budding in the Southwest and who suggested that ponderosa pine may have a particular tendency toward epicormic branching, have since found these extraordinary growths on pines in other regions. As a matter of interest, one or more of the forms of substitute shoot development that has been described for ponderosa pine has also been observed in the southwestern yellow pines, Chihuahua (*Pinus leiophylla*) and Arizona (*P. arizonica*).

The suggestion that this tendency may be peculiar to 3-needled pines has also been received. As regards white pines, a few limber pines (*P. flexilis*) occur in the same general region where ponderosa pine was studied. They produce extraordinary growths when injured quite as readily as do pon-

FIG. 8. Extraordinary growths on a young limber pine, which shoots opened from needle-bundle buds, as evidenced by the five needles (characteristic of limber pine) at the base of each shoot.

derosa pine (fig. 8). Such growths have been found on Mexican white pine (*P. strobiformis*) also. So far as is known, there are no other records of this growth phenomenon in white pines.

In ornamental plantings in the Southwest, evidence of recovery from injury through development of needle-bundle buds has been observed on several different unidentified young 3- and 5-needled pines and on the 2-needled Scotch pine (*P. sylvestris*).

Factors causing injury, in relation to recovery

Destructive factors, the injuries they do, and the circumstances under which injury takes place will be discussed in other presentations for publication—those presenting the results of the grazing-timber reproduction study previously mentioned. Only summarized statements of findings which show the influence different factors have on extraordinary bud development will be given here.

Tip moths

Tip moths deposit eggs on the current growth of pines during the early period of shoot elongation. The larvae, on hatching, eat into and sometimes through the entire length of a shoot. Badly affected shoots die; others die in part (fig. 9). In any event, moth injury influences the chances and processes of recovery somewhat differently than mechanical injury, as from browsing. If only the tip part of a moth-injured shoot dies, as is frequently

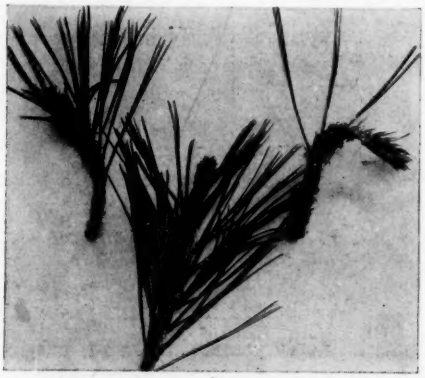

Fig. 9. Three tip-mothed ponderosa pine shoots. Left, only the tip was injured, but all except the lower four or five bundles were affected, and they show no evidence of bud development. Right, entirely dead (the three lower needle bundles developed before the larvae reached them). Middle, shoot, nearly full growth, damaged only at tip and side. Such a shoot that attains nearly full growth before its tip dies and that has many unaffected leaves does not, as a rule, develop needle-bundle buds.

the case, needle-bundle buds may or may not open. In contrast to other injuries, the effect of moth injury is progressive and cumulative; it may possibly be toxic. Larvae may be active throughout most of the period of shoot elongation, and any foliage leaves that survive may become stunted and weak. When living but weakened leaves are left on a shoot, no needle-bundle buds usually develop.

The stage of development of pine shoots at the time moth larvae become active may vary considerably from year to year. In some years when moths fly early and their larvae attack shoots during the early period of elongation, the chances of recovery through opening of needle-bundle buds is seriously affected. Such years were 1927 and 1928, when examinations of thousands of moth-injured shoots showed needle-bundle buds on less than 1 per cent. In years when moths fly late, the vigorous and rapid shoot growth of mid-season seems to counteract the damaging effects of larvae and many injured shoots have only destroyed tips or sides, in which event a large percentage of the shoots with undamaged foliage leaves develop needle-bundle buds.

Where shoots are destroyed to the extent that no needle-bundle buds open from them, whorl buds commonly develop on the old stems below the larvae-destroyed leading growth. However, where substitute shoots from whorl buds are moth-injured, the chances of additional substitute growth are greatly reduced, because of the lack of other dormant buds from which such growths might open. The cumulative effect of injuries and the small percentage of recovery through needle-bundle buds indicate how serious tip-moth damage may become. Some tip-mothed plants (in places nearly all of them) on large areas, particularly in warmer parts of southwestern pine forests, remain bushy and deformed until they reach a height beyond which moths ordinarily fly. However, most tip-mothed trees apparently do recover ultimately.

Tip moths are particularly destructive to small plants during the first few years of long-shoot growth; for their shoots are small and any affected shoot usually is completely destroyed. Small plants soon become bushy on account of recurring injury to leading growth and regrowth through opening of numerous substitute buds.

Browsing animals

Browsing animals took only young shoots and, excepting deer, browse in about the same manner and during the same period.

Any browsing of consequence by cattle, sheep, and antelope occurs during dry periods. It begins in early summer on the most advanced shoots after they have made substantial length growth, but while their needles are still short, as those in A of Y figure 2. Browsing continues throughout the early summer dry period, but ceases with the first summer rains, regardless of the

stage of shoot development. During occasional years of few and irregular summer rains, browsing recurs to some extent during any extended dry period between rains. In most years the period of pine browsing extends from about mid-June to early July.

The earlier shoots are browsed during the annual growth period, the sooner substitute shoots and buds appear and the more pronounced is their development. Where leading growth is browsed off after shoot elongation is complete, either needle-bundle or old-stem buds may fail to develop, at least to any noticeable degree during the same year. The growth of buds that are but slightly stimulated is tardy the following year and the shoots that open from them sometimes are weak and persist as side branches only. Thus, late injury and consequent weak substitute growth tends to produce the greater temporary deformities in the plant.

Deer, in contrast to other animals studied, may destroy terminal buds soon after shoot elongation begins in spring. On parts of experimental ranges where deer congregated and where fresh deer tracks but no sign of other browsing animals could be found, shoots were browsed in May during the early stages of growth before livestock were on these ranges.

Similar findings and general observations over a period of years showed that deer browse pine shoots earlier than other animals. As the season advanced, deer-browsed shoots became difficult to identify because livestock were then taking shoots, and deer scattered from the particular areas where spring damage was found. However, observations indicate that deer continue to take shoots throughout the period when other animals browse. The most conclusive proof of this was found in the Kaibab country north of the Grand Canyon. Here deer have browsed shoots just as severely as cattle and sheep ever do and also continue browsing throughout the period of shoot elongation, or at least until the coming of summer rains.

Where deer peel all the developed terminal buds from a stem, the whole shoot, which is then very short, ordinarily is destroyed. Under such circumstances old-stem whorl buds develop rapidly and soon may form extraordinary growths that closely resemble ordinary shoots (fig. 10). Likewise, if the tip of a very small shoot is so taken as to leave one or more of the then slightly developed short shoots, needle-bundle buds may develop and grow into large shoots the same year.

Test of injury and recovery

Individual plants under controlled conditions were injured mechanically and compared with uninjured plants in order to test the effect of different kinds, combinations, and degrees of browsing where given injuries were repeated on the same plant over a period of years.

Incidental to the purpose and main findings of this study, it was observed that extraordinary budding took place just as it did on browsed plants, so

FIG. 10. Young substitute shoots (early August) from whorl buds in which growth was stimulated through loss of terminal buds before shoot elongation began in the spring (late May), showing how such early-growing extraordinary growths resemble ordinary shoots. Deer peeled the swelling buds, leaving the stubs and scales (*a*).

long as shoots were injured at about the same stage of growth and in a similar manner as was done by browsing animals. In short, needle-bundle, whorl, and secondary buds developed when young annual growths were removed mechanically.

Among these mechanically injured plants, the extent of current development of substitute buds or shoots also bore a definite relation to the stage of normal shoot development when injury occurred. To wit, the best buds were produced where shoots were removed before elongation was completed; and among well-developed shoots, only those with immature foliage leaves (needles continue to grow after full shoot length is attained) produced buds the same year. Plants from which leading shoots were removed when both shoots and foliage were fully developed failed to produce noticeable evidences

of buds. On some of these, buds appeared the following year but sometimes failed to open shoots. This shows that substitute buds are awakened most readily and some develop only during the periods when the plant is normally expending stored energy in the production of annual shoot growth. It would seem to indicate that the extent of development of current substitute growth is determined by the amount of unexpended annual growth energy in the plant.

Another interesting and significant observation made on both mechanically injured and browsed plants, and which was demonstrated again and again by the different kinds of growths, is the strong tendency for substitute buds on leading growths to produce leading growths (fig. 11). In most instances shoots from needle-bundle buds gain the lead even where some

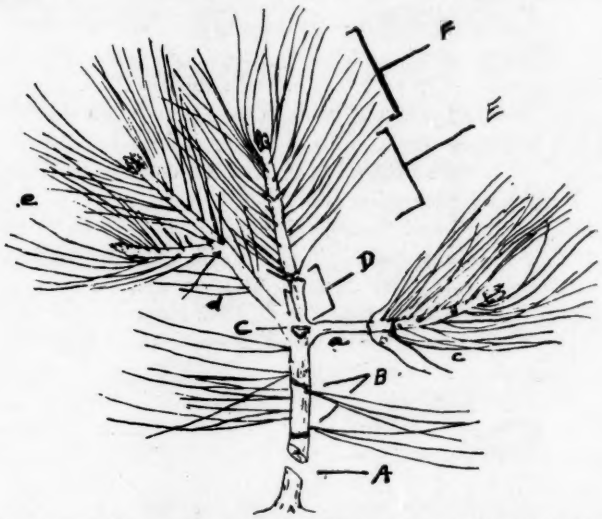

FIG. 11. A many-times browsed ponderosa pine seedling (12 in. high, 10 years old), showing how injured leading shoots tend to produce leading shoots, and how stem length may represent a succession of stubs. A, unsketched part of main stem (3 in.) which carried evidence of several injuries and substitute growths. B, rind creases, evidence of substitute growth following injury. C, dead stub of browsed 1926 growth. D, stub of 1927 shoot from a whorl bud on 1925 growth (stem), following removal of the one leading shoot (C) in 1926. E, stub of 1928 growth from a needle-bundle bud. (Note the peculiar needles at the base of this and other extraordinary needle-bundle growths.) F, 1929 shoot from a needle-bundle bud. The left branch shows uninjured 1927 and injured 1928 growth (*d*). The 1927 shoot was from a whorl bud, as D. The browsed 1928 shoot gave rise to two needle-bundle buds from which opened the 1929 shoots (*e*). Branch to right: *a*, stub of 1927 growth which was produced and injured as D; *b*, stub of 1928 shoot that opened from a needle-bundle bud; *c*, 1929 growth from a needle-bundle bud.

uninjured shoot or branch assumes control for one or more years; and buds on leader stubs are more apt to produce leaders than buds on laterals where both are present. In the absence of needle-bundle buds, uppermost whorl buds (any of which might have been the leading growth in the past) gain the lead when stimulated to make growth. The uppermost bud, either needle-bundle or whorl, on the main stem where substitute buds open on both main stem and branches, is most apt to produce leading growth. The tendency of leading growth to retain the lead is also influenced by the energy the plant has expended in making shoot growth at the time injury occurred. As evidence of this, practically all exceptions to the rule that buds on leading growths produce leading growths were found on plants that were injured too late in the growth period to develop vigorous buds or shoots.

Substitute growths and recovery of injured pines

This discussion of substitute budding naturally leads to consideration of the bearing these recovery processes have on reforestation. How dormant buds are stimulated and how substitute growths take the place of lost leading growths have been described. What peculiar influence, if any, substitute growths may have on the future life of trees beyond the pole stage can be determined only after a long-time study.

Repeatedly browsed and tip-mothed young pines may be temporarily as bushy as the most common hedge plants. Particularly where juveniles are injured, many substitute shoots are produced for each normal growth that is lost. When such shoots in turn are injured, numerous others open and persist within the margin of the older branches, although any current shoot may be tip-mothed and when it extends beyond the old branches may be browsed. Thus hedged plants build up branches and foliage and gradually gain height. They function and grow through opening of extraordinary buds and shoots which persist through the protection that hedged growth affords (fig. 12). Tests, on the basis of comparison with normal plants of the same age, showed that hedged plants have about as much foliage, both according to number and surface area and total weight of needles as the normal plants, and also a good root system.

The behavior previously described is characteristic of severely injured plants, but most seedlings on any large range, even in districts where browsing is apt to occur, are not injured every year and never become bushes from browsing, although some plants on areas of animal concentration on the same range may be injured for several years in succession. Furthermore, conditions peculiar to some districts where browsing occurs vary so greatly that many pines may be browsed in some years and few in others. Where for any reason plants escape browsing on some years, they open exceptionally large leading shoots the same years and thus maintain reasonably normal growth over a period of years (fig. 13).

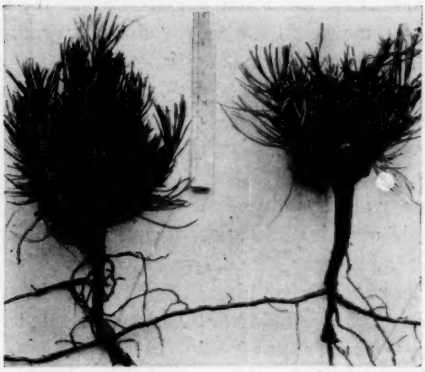

FIG. 12. Two pine bushes about 6 inches high and 9 years old, hedged as the result of many browsing and tip-moth injuries. They show how injured young plants continue growth through the opening of many substitute shoots, and how the stubs of old shoots furnish some protection to the new shoots. Plant on right was severely browsed the year it was photographed; that on left developed a 2-inch leader.

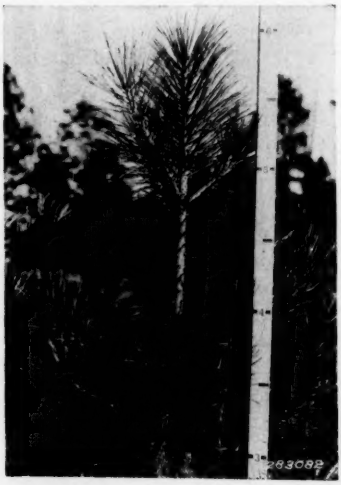

FIG. 13. Accelerated growth made by a formerly short, bushy ponderosa pine. Three-fourths of the total height growth of this plant was made in 3 years, despite the fact that the leading shoot was removed once during that time. On the year following this injury, the long unbranched part of the main stem opened from a needle-bundle bud. The many branches (bush) on the first base foot of the stem died when accelerated growth supplanted the need for the 4- to 7-year-old foliage which had persisted on these branches.

Accelerated growth of hedged plants continues for several years following release from injury, some substitute shoot ordinarily assuming the lead, and, in 3 or 4 years, extending a heavy normally straight stem far above the limits of the hedge from which it sprang (figs. 5, 12, and 13). Almost as soon as such a new main stem has clothed itself with branches and foliage that is in accord with normal plants of its size, the hedged branches at its base cease to function, and die.

The extraordinary ability to produce substitute buds and shoots accounts for the power ponderosa pine has to recover from shoot injury. If it were not for this ability to bud, areas having peculiar conditions that induce animals to browse pines probably could not be grazed by livestock or large game animals at all without jeopardizing future forests. Rodents and tip moths certainly would prevent reforestation in places. The same may be said of the past concerning conditions under which older or mature forest stands developed. When considering the districts in central Arizona below the Mogollon Rim, where nearly all young pine trees up to large pole size are injured year after year by tip moths, one wonders whether many, or even any, of the mature trees escaped injury when they were young. It is fortunate that browsing and tip moth injury affect only current shoot growth and so allow for the development of needle-bundle and dormant whorl buds, and that such injuries occur during periods of normal shoot production when substitute buds are most readily awakened, otherwise injuries would result in much more serious consequences than they do.

But little may be said concerning grazing and timber reproduction relationships in this discussion of recovery processes. The following results are recorded as they indicate the importance of the fact that pines replace lost shoots through a number of different processes of substitute growth.

Of the 2139 ponderosa pine seedlings (about 7 years old in 1927) on 31 plots so scattered as to represent conditions on a 24,000-acre area, 1484, or 69 per cent., were browsed one or more times, some every year during the 9-year period from 1927 to 1935. Of the browsed plants, 2.8 per cent. died. Small rodents injured 171, or 7.9 per cent. of the total number of trees on the same plot areas. Of the 171 injured trees, 127, or 74 per cent., died. Most of the rodent injury consisted of barking or cutting off of main stems.

Of the 5172 recorded seedlings that survived the first 2 months, when mortality from natural causes was tremendous, a total of 74, or 1.4 per cent., were killed by browsing animals and 382, or 7.3 per cent., by rodents. Neither these data nor the statements regarding recovery processes should be interpreted to mean that overgrazing or poor range management may be disregarded. The results of the range-timber reproduction study, which will be presented in future publications, show grave dangers in such practices.

It is well known that trees, particularly hardwoods, when grown from stump and trunk sprouts sometimes are small as compared with seed-produced trees, and may take on bushy or coppice form. No such tendency has been observed in young ponderosa pine poles that developed from extraordinary buds. In this connection, the processes of recovery which have been described take place during the early life of the plant, and substitute shoots actually take the place of lost shoots, rather than form the source of new growth that is superimposed on a part of an old tree, as is often the case with dwarf and coppice growths from sprouts.

SOUTHWESTERN FOREST AND RANGE EXPERIMENT STATION
TUCSON, ARIZONA

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UREASE DISTRIBUTION IN *SOJA MAX*[1]

SAM GRANICK[2]

(WITH FIVE FIGURES)

Introduction

In this account, the distribution of urease, as studied by histological and quantitative methods (**10**), will be discussed under the sub-headings of the various plant structures.

The activity of the enzyme is expressed in U.U., a urease unit, which is the quantity of urease that will produce 1 mg. of NH_3 per minute when acting upon urea under the conditions employed in the analyses.

Soja max var. Manchu is an upright leafy branching annual legume. The appearance of the plant is much affected by the conditions under which it is grown. In the field, the plants produce a luxuriant growth, forming large leaves, stout, woody stems, numerous pods, and branching is frequent. The plants grown in the greenhouse at Ann Arbor were three feet tall at maturity and rarely branched. The same variety of bean, grown at Woods Hole, Massachusetts, in wooden boxes in soil 6 inches deep, attained a height of only 8 inches and bore 1 to 2 pods per plant. Unless otherwise stated, the determinations described in the following pages were made on plants grown in the greenhouse at Ann Arbor. The reader is referred to the first paper of the series (**10**) for details as to sampling of material for analysis, etc. The distinctive taxonomic description of *Soja max* var. Manchu is given by ETHERIDGE, HELM, and KING (**6**).

Investigation

COTYLEDONS OF THE GERMINATING SEED

The seed of the soy bean, because of its commercial importance, has been made the subject of numerous chemical investigations. Since data on the chemistry of the seeds were available, it became interesting to attempt to find a correlation between urease and the various chemical substances present in the seed. As was to be expected from the fact that urease is a globulin, the changes of urease were found to be related directly to the general changes in protein content. The cotyledons of jack bean contain much protein and starch. The cotyledons of soy bean are notable for their high protein and fat content, and at maturity contain less than 0.5 per cent.

[1] This is the third of a series of papers on urease distribution in *Canavalia ensiformis* and *Soja max*. For more extensive data and discussion, the reader is referred to the original thesis available at the University of Michigan library.

[2] Newcombe Fellow in plant physiology.

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starch. Both kinds of seeds possess the highest reported urease activity of any materials yet found. SUMNER (**24**) estimates that soy beans contain about 0.01 per cent. urease by dry weight.

The histological urease reagents showed that urease was highest in content in the germinating cotyledons. As germination proceeded there was a gradual decrease of the enzyme (figs 1, 2). In the 8-day-old seedlings,

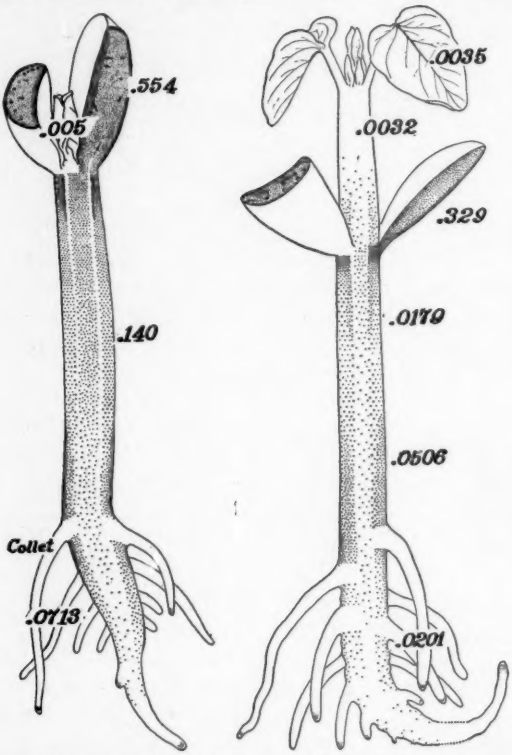

FIG. 1. Composite representation of the urease distribution in the various structures of soy bean seedlings 8 and 15 days old. The number opposite each region of the seedling expresses the number of urease units per gram of fresh weight of that region, as determined by the quantitative method. Stippling indicates the relative urease concentrations as revealed by the histological methods.

the enzyme appeared to be most concentrated at the periphery of the cotyledons, that is, in the subepidermal layers. Urease had diminished markedly in the center of the cotyledons. Some large parenchyma cells gave a stronger reaction for urease than others. The rate of depletion of urease in these

cells showed no evident relation to their proximity to the vascular bundles. The 3 to 4 layers of palisade cells gave a somewhat stronger urease reaction than did the other parenchyma cells. The vascular bundles showed no detectable urease. The content of urease of the bundle sheaths was neither higher nor lower than that of the neighboring parenchyma cells.

Because the chlorophyll content of the outer cells, to some extent, masked the urease reaction, observations were also made on seedlings germinated in

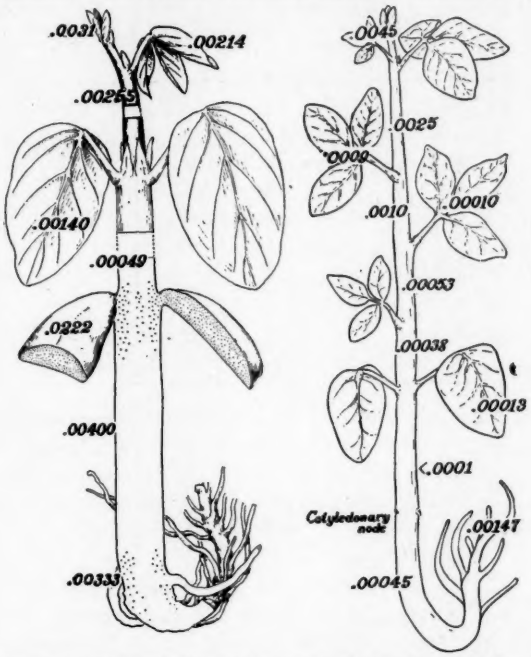

FIG. 2. Composite representation of the urease distribution in the various structures of soy bean seedlings 31 and 52 days old. Numbers have the same meaning as in figure 1.

the dark. There was a more rapid depletion of storage substances from cotyledons of etiolated than non-etiolated seedlings. The urease distribution in the etiolated cotyledons was similar to that in the green cotyledons, except that urease diminished more rapidly in the etiolated cotyledons.

In the 16-day-old seedlings the urease distribution in the cotyledons was similar to that in the 8-day-old plants, except that the total concentration of urease had decreased. The parenchyma cells of the under surface gave a somewhat stronger urease reaction than did the palisade or other parenchyma cells.

In the 30-day-old seedling the cotyledons, which were by now falling off, were yellow, flaccid, and shrivelled. Determinations on these cotyledons generally showed a low urease content.

TABLE I

UREASE CONTENT OF THE COTYLEDONS OF SOY BEAN SEEDLINGS

TEMPERATURE DURING GERMINATION	DAYS AFTER PLANTING	FRESH WT. IN GM. PER COTYLEDON	DRY WT. IN GM. PER COTYLEDON	U.U. PER GM. FRESH WT.	U.U. PER COTYLEDON
°C.	*days*	*gm.*	*gm.*	*U.U./gm.*	*U.U.*
27	0	0.0803	0.0704		
	1	0.1606	0.0693	2.12	0.341
	2	0.1716	0.0688	2.96	0.507
	4	0.1885	0.0554	2.35	0.442
	6	0.306	0.0414	1.48	0.453
20	3	0.176		1.80	0.317
	7	0.215		0.755	0.162
	8	0.222		0.554	0.123
	15	0.356		0.329	0.117
	31	0.263		0.0222	0.00844

A quantitative study was made of the changes in the quantity of urease in the cotyledons at various stages of germination. The changes in urease content taking place at temperatures of 20° and 27° C. during the process of germination and early development of the seedling are shown in table I. These changes are complicated by various factors of which the following are suggested:

1. There will be a decrease of urease as the proteolytic enzymes split the proteins and urease into smaller, more soluble transport products. The urease content will depend then on the activity of the proteolytic enzymes and the rate of transport of their products away from the cotyledons and into the developing tissues.

2. Proteins may be in a storage phase *i.e.*, in aleurone grains, etc. If some urease is present in this state it cannot be detected, for the enzyme must be in a dispersed state in order that urea molecules may come in contact with it (**10**). Therefore, it is necessary to consider the rate at which dispersal of the stored urease occurs.

3. There is also a possibility that some urease may be synthesized in the cotyledons during germination.

One may consider that during the first days of germination the storage proteins are dispersed and come into solution in the form of soluble proteins at a rate which is more rapid than the rate at which the soluble proteins are

hydrolyzed. The dispersal of proteins is faster at 27° than at 20° C., since water is taken up more rapidly by the soaking seeds at the higher temperature. At 27° C. the urease activity of the cotyledons is greatest after two days germination. The amount of protein being dispersed soon decreases owing to the fact that little storage protein is left; while the dispersed or soluble proteins continue to be hydrolyzed at a rapid rate. Thus a decrease in the amount of soluble protein will take place, appearing as a decrease in urease activity.

Radicle

The *radicle* in the 2-day-old seedlings, was about 1.3 cm. Cross sections were made through various portions of the radicle and their urease content was determined. Sections through the tip of the radicle showed that the plerome contained a high percentage of urease; the periblem and dermatogen gave only a faint reaction. Sections through the middle of the radicle showed that the epidermis and tetrarch xylem contained no detectable urease; the pith was faintly positive; the cortex was strongly positive. There seems to be a tendency for urease to disappear more rapidly from the pith than from the cortex. In the jack bean there appears to be a tendency for urease to decrease more rapidly in the cortex than in the pith. In general, however, the distribution of urease in the radicle of soy bean is similar to that in the jack bean.

Examination of the collet region of 8-day-old seedlings showed a high urease content in the wide cortex area and relatively little urease in the pith (fig. 1). The endodermis, the cambium, and the phloem and the xylem of primary and secondary origin appeared to contain no urease. (The difficulty in judging the slight urease content of a group of cells, adjacent to

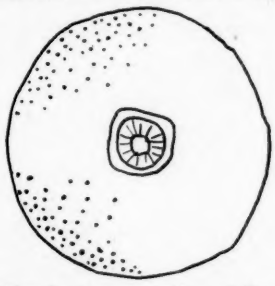

Fig. 3. Urease distribution in a cross section of the collet region of a 39-day-old seedling.

another group of cells containing much urease, has been discussed previously for the collet region of the jack bean.) The bursting of the secondary roots through the cortex causes some of the cortical parenchyma cells at the break to become slightly brownish and to increase in alkalinity to pH 7, or somewhat higher. The young secondary roots were separated from the main root and

tested by themselves. Not more than 50 per cent. of these roots gave positive indications with the urease reagent, and these showed urease to be present at the growing points and nowhere else. Evidently the method is not sensitive enough to detect the low activity of all of these roots. There appeared to be no correlation between the position on the collet axis from which the secondary root arose, and its urease activity at the growing point.

After 16 days urease was still present in the small outer cortical cells. The innermost cortical cells and the stele contained no urease. The collet region loses its urease activity more slowly than any other region. Urease could still be detected in the collet in a 30-day-old seedling but the enzyme was delimited to isolated portions of the outer cortical cells (fig. 3). In the 46-day-old plant no urease could be detected in the collet.

Root

In the 8-day-old seedling the root is about 7 cm. long. Urease was found to be highest in content in the region just below the collet and to decrease rapidly down the root until within 2 cm. of the tip where it could no longer be detected (fig. 1). The outer cortical cells contained the highest amount of urease at the collet region, but as one descended along the root, the urease reaction became diffuse in the cortex. No urease was present in the stele. At the growing point of the root there was a slight amount of urease which decreased so rapidly that it could not be detected in the elongation region. Generally the primary root tips at this stage all gave the urease reaction, in contrast to the secondary root tips which, on the whole, had a lesser urease content and gave indications of urease in less than 50 per cent. of the secondary roots examined. Very little urease was found in the 15-day-old seedling, in the region of the root just below the collet. Urease therefore decreases quite rapidly with age in the roots, except at the growing points.

TABLE II

Urease content of the root

Days after planting	Structure	Fresh wt.	U.U. per gm. fresh wt.	U.U. per structure
days		*gm.*	*U.U./gm.*	*U.U.*
1	Embryo minus cotyledon	0.0122	2.66	0.0324
3	Embryo minus cotyledon	0.0975	0.517	0.0505
4	Root and collet	0.105	0.0973	0.0102
8	Root and collet	0.131	0.0713	0.0093
15	Root and collet	0.274	0.0201	0.0055
31	Root and collet	0.419	0.0033	0.0013
52	Root and collet	1.36	0.0014	0.0020

In table II are shown the changes in urease content that take place in the root with age. There is a rapid decrease of urease activity per gram of

fresh weight; a rise of total urease per structure to a maximum two days after planting and then a decrease of total urease after that day. Even though the roots have increased rapidly both in weight and number of secondary roots, the total urease content decreases. As will be shown later, this rapid decrease in urease content may be related to the fact that the elongation region of the root is a relatively short one, and the elongating cells mature rapidly.

Hypocotyl

In the 8-day-old seedling urease had already begun to decrease sufficiently so that differences in concentration could readily be noted in the hypocotyl with the histological urease reagent (fig. 1). The enzyme content was greatest at the cotyledonary node and at the collet. Urease was found only in the parenchyma cells of cortex and pith; never in the cambium or its derivatives. The urease content of the pericyclic cells could not be determined with certainty, although there are indications that these cells do contain urease. Sections through the cotyledonary node showed the pith to contain slightly less urease than the cortex. The enzyme decreased rapidly in the pith from the upper to the lower part of the hypocotyl. At the top of the hypocotyl there appeared to be more urease in the cortical parenchyma cells adjacent to the stele. There was a gradual decrease in urease content in the cortex from the top of the hypocotyl to the lower end, but, on nearing the collet, urease appeared to become more concentrated in the outer smaller cortical cells.

In the 16-day-old seedlings the differences in enzyme content of the various cells had become more marked. The hypocotyl, as seen in cross section just below the cotyledons, still gave strong urease reactions. Owing to elongation there was a mechanical thinning out of the enzyme at this stage, taking place most rapidly in the upper two-thirds of the hypocotyl. In the remainder of the hypocotyl urease became restricted to the outer cortical cells and little of the enzyme was to be found in the pith.

In the 30-day-old seedlings (fig. 2) urease was detected only at the cotyledonary node, and just within the collet region; in both these instances urease was restricted to the outer cortical parenchyma cells. In the 50-day-old plants (fig. 2) no urease could be detected in any portion of the hypocotyl.

Within a period of seven days the hypocotyl had increased from 3 to 60 mm. It will be noted from the data of table III that after the eighth day of germination primary growth and elongation of the hypocotyl had practically ceased. Even before this time the maximum total urease content of the hypocotyl had been attained. The phases of primary growth and maturity overlap. The quantitative data of table III and the results obtained with the histological reagents showed that urease was not only being me-

TABLE III

UREASE CONTENT OF THE HYPOCOTYL

DAYS AFTER PLANTING	STRUCTURE	FRESH WT.	U.U. PER GM. FRESH WT.	U.U. PER UNIT STRUCTURE
days		*gm.*	*U.U./gm.*	*U.U.*
1	Embryo minus cotyledon	0.0122	2.66	0.0324
2	Embryo minus cotyledon	0.0975	0.517	0.0505
8	Hypocotyl 6 cm.	0.233	0.140	0.0326
15	Hypocotyl 7 cm.	0.404	0.0347	0.0140
	Upper half	0.194	0.0179	0.00347
	Lower half	0.210	0.0506	0.0106
31	Hypocotyl	0.434	0.00400	0.00173
52	Hypocotyl	0.500	0.00045	0.00020

chanically thinned out, but that already in the late stages of primary growth the enzyme was actually being inactivated or catabolized so that the total urease content of the hypocotyl decreased with further age.

STEM

The apical meristems possess a urease activity that is too low to be studied with the histological reagent. From quantitative data there is reason for believing that there is no difference in urease distribution between the apical meristems of the soy bean and the jack bean, except the difference in urease concentration.

The epicotyl or second internode[3] is considered apart from the other stem internodes because of its proximity to the cotyledonary node. In the 9-day-old seedling the epicotyl is about 5 mm. long. Cross sections through the tip of the epicotyl, where the first leaves arise, showed urease to be present in the outer cortical parenchyma cells and also faintly in the pith.

In the 16-day-old plant (fig. 1) the epicotyl showed the presence of urease only in the region close to the cotyledonary node; the urease reaction had diminished considerably in intensity. No cells except the parenchyma cells of the cortex, and more faintly the pith, gave a positive test with the urease reagent. In the 30-day-old plant no urease could be detected in the epicotyl.

The quantitative data for the urease content of the epicotyl are given in the table IV. The figures show that the most rapid elongation occurred between the eighth and fifteenth day, during which time the total urease per

[3] For convenience the hypocotyl has been designated as the first internode although this is not technically correct according to the terminology of the morphologist. The epicotyl is considered as the second internode and the other internodes are numbered successively upwards.

structure increased markedly, while the urease activity per gram of fresh weight decreased slightly. Although elongation still occurred after the fifteenth day, it was relatively slight. The phases of primary and secondary growth overlap each other. It may be concluded that the total urease content is at a maximum during the period of the most rapid elongation of the cells of primary growth and thenceforth urease begins to decrease in activity.

TABLE IV

UREASE CONTENT OF THE EPICOTYL (SECOND INTERNODE)

DAYS AFTER PLANTING	STRUCTURE	FRESH WT.	U.U. PER GM. FRESH WT.	U.U. PER STRUCTURE
days		*gm.*	*U.U./gm.*	*U.U.*
8	5 mm. plumule	0.0116	0.005	0.00005
15	2nd internode 6 cm.	0.118	0.0032	0.00030
31	2nd internode 9 cm.	0.325	0.00049	0.00015
52	2nd internode 10 cm.	0.509	0.0001	0.00005

Examination of sections made of the other internodes, above the second, and tested with the urease reagents indicated that the amount of urease present even in the youngest internodes was too small to be detected.

Quantitative determinations were made of the urease content of the internodes above the second and the data are shown in table V. Although growth in length is not exactly the same for each internode, (the weight of the sixth internode, for example, being greater than the weight of any older internode) the changes of urease with age are still sufficiently marked to be

TABLE V

UREASE CONTENT OF INTERNODES OF A 52-DAY-OLD PLANT

STRUCTURE	FRESH WT.	U.U. PER GM. FRESH WT.	TOTAL U.U. PER STRUCTURE
	gm.	*U.U./gm.*	*U.U.*
7th internode including leaflets and apical bud	0.064	0.0031	0.00018
6th internode	0.250	0.0025	0.00062
5th internode	0.223	0.0010	0.00020
4th internode	0.171	0.00053	0.00009
3rd internode	0.241	0.00038	0.00008

clearly recognized. These changes in urease content are similar to those undergone by other plant structures. The urease activity steadily decreases with age, the oldest internodes having the lowest urease activity per gram of fresh weight. The total urease per internode is highest in the sixth

internode. This internode has just finished its maximum primary growth. From that time, the total urease content diminishes with the maturation and senescence of the internode.

Leaves

In the 1-mm. first foliage or plumule leaves of the ungerminated soaked seed urease was found in all the cells except the primary xylem. The histological reagent also showed that urease decreased rapidly as the leaf expanded.

The data on the urease content of the first pair of foliage leaves of various ages are presented in table VI. The figures in the table indicate the same trend for changes in urease content as was found in the structures previously examined *i.e.*, a decrease of urease activity per gram of fresh weight; and for the total urease content per structure, at first a rise to a maximum which coincides with the period of maximum elongation, then a slow decrease after primary growth has ceased.

TABLE VI

Urease content of the first pair of foliage leaves

Days after planting	Structures	Fresh wt.	U.U. per gm. fresh wt.	U.U. per structure
days		*gm.*	*U.U/gm.*	*U.U.*
8	Plumule—5 mm.	0.0116	0.005	0.00005
15	Leaves—3 cm. and petioles—4 mm.	0.170	0.0035	0.00060
31	Leaves—4.5 cm. and petioles—1.5 cm.	0.387	0.00140	0.00053
52	Leaves—5 cm.	0.405	0.00013	0.00005

Sections through the embryonic compound leaves in the apical buds above the second node gave no indication of the presence of urease when the histological method was used. The urease content in the meristematic tissues of the stem, and in the leaf primordia is too low to be detected by this method.

Determinations were made on the trifoliate leaves of soy bean plants grown in the field during the summer, and on others grown in the greenhouse during the winter. The plants grown in the field were harvested at the stage when they possessed pods that were almost mature. The quantitative data (table VII) show the same general trend of urease metabolism as do the plumule leaves. The leaves of the field-grown plants were larger, weighed more, and possessed a urease activity which was maintained at a high level even after primary growth had ceased. The maximum size of the field-grown leaflet was 11 cm. as compared to 6 cm., the maximum size of the

TABLE VII

UREASE CONTENT OF COMPOUND LEAVES

LEAF	FRESH WT.	U.U. PER GM. FRESH WT.	U.U. PER LEAF
	gm.	*U.U. gm.*	*U.U.*
Greenhouse-grown plants 52 days old			
7th node ..... 3 cm. leaflets	0.355	0.0045	0.0015
6th node ..... 6 cm. leaflet, 7 cm. petiole	0.777	0.0013	0.0010
5th node ..... 6 cm. leaflet, 7 cm. petiole	0.673	0.0009	0.00060
4th node ..... 5 cm. leaflet, 7 cm. petiole	0.463	0.0001	0.00004
Field-grown plants with nearly mature pods			
16th node ..... 4 cm. leaflet, 2 cm. petiole	0.414	0.00517	0.00214
15th node ..... 7 cm. leaflet, 5 cm. petiole	1.03	0.00467	0.00454
14th node ..... 9 cm. leaflet, 8 cm. petiole	2.28	0.00409	0.00932
13th node ..... 11 cm. leaflet, 10 cm. petiole	3.31	0.00346	0.0114
12th node ..... 11 cm. leaflet, 10 cm. petiole	3.11	0.00264	0.00821
11th node ..... 11 cm. leaflet, 13 cm. petiole	3.98	0.00238	0.00946
10th node ..... 11 cm. leaflet, 13 cm. petiole	3.16	0.00227	0.00718
9th node ..... 9 cm. leaflet, 8 cm. petiole	2.34	0.00294	0.00688
8th node ..... 8 cm. leaflet, 9 cm. petiole	2.09	0.00258	0.00539

greenhouse-grown leaflet, and the weights of each were respectively 3.98 and 0.777 gm. The leaves of the plants grown in the field had a urease activity of 0.005 to 0.002 U.U. per gram of fresh weight, whereas the urease activity of leaves from greenhouse-grown plants was 0.004 to 0.0001 U.U. per gram of fresh weight. If it is assumed that urease activity follows changes in soluble protein content, then it might be said that the greenhouse-grown leaves behaved as if there was a lack of available carbohydrate in the plant for maintaining a high level of protein nitrogen, the depletion of urease and protein taking place rapidly when the leaves had finished expanding because of the drain of carbohydrate and non-protein nitrogen from them into the growing points. However, even under the different environmental conditions of summer and winter growth, expressing themselves in such a wide divergence of growth in green weight and in urease content, the same general scheme of urease metabolism in the leaves was observed as had been noted for the other structures of the plant, namely, that the highest urease

activity was present in the youngest leaves and decreased with age of the leaf; and the total urease content increased during primary growth and then decreased.

Flower and fruit

The urease content of the flower primordia, the flower, and the pod tissues is below the concentration that is detectable with the histological reagents. The quantitative changes of urease content in the developing pods may be noted from the data in table VIII. The course of the change in urease activity

TABLE VIII

Urease content of pod

Structure	Fresh wt.	U.U. per gm. fresh wt.	U.U. per unit structure
	gm.	*U.U./gm.*	*U.U.*
Young flowers and buds	.....	0.006	.....
Pod (contained 3 mm. seeds)	0.1490	0.0051	0.00076
Pod (contained 5 mm. seeds)	0.2010	0.0042	0.00084
Pod (contained 9 mm. seeds)	0.2518	0.0025	0.00063
Pod (contained 11 mm. seeds)	0.371	0.0011	0.0004

is similar to that found for other tissues, that is, there is a steady decrease of urease activity per gram of fresh weight; and an increase and then a decrease in the total content of urease per pod.

The plant as a unit

A survey of the changes in the total urease content of the developing seeds and of the seedlings of soy bean is given in a graph (fig. 4). Along the abscissa the relative age of the bean or plant is represented. On the ordinate is plotted logarithmically the total urease values. It may be observed from this graph that the urease content increased almost ten thousandfold from the youngest seed examined (3.5 mm. long) to the mature seed (13.5 mm. long). Although in the last stages of maturity the seeds were beginning to dry, decreasing in length from 13.5 to 12 mm., and in water content from 63.3 to 48.6 per cent., the urease content still increased. Finally, at the last stage, when the seed had shrunk to 9 mm. and the water content decreased to 19.4 per cent., the urease content had decreased to approximately one-sixth the value of the previous stage.

The completely mature and dry seeds were then germinated. Those planted at 27° C. showed an increase in urease content which reached a maximum at the second day and then began to decrease. Plants grown at 20° C. had a lower level of total urease per plant. There was a rapid decrease of total urease per plant from the fifteenth day.

As with the jack bean, the curves illustrating the changes in total urease content per seed or per plant really indicate the changes of urease taking place in the cotyledons, since these structures contain most of the enzyme. In the developing seed, the cotyledons, especially in the later stages, consti-

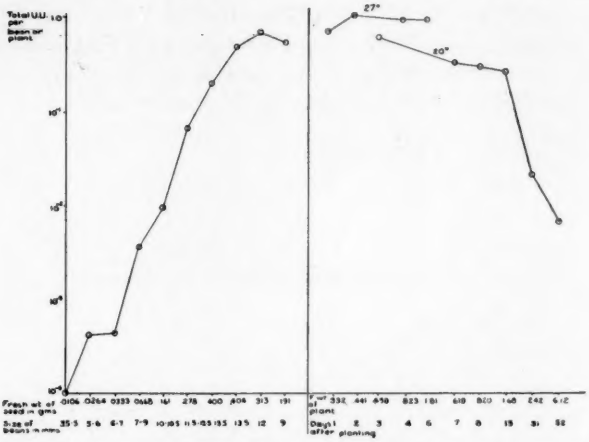

FIG. 4. The graph represents the total urease contest of the developing beans, or of the seedlings of soy bean, at different stages of growth. Along the ordinate, total U.U. values are plotted logarithmically; along the abscissa, relative ages of the beans or seedlings are represented.

tute over 90 per cent. of the dry weight of the seeds. As the seeds increase in size, urease increases very rapidly. In the last stages when the seeds begin to dry (as indicated by the pods turning yellow, the hila of the seeds changing from green to pink then to purple and finally to black, and the cotyledons from a green to a yellow-green and finally to yellow) urease increases and finally decreases. This decrease in urease activity is interpreted as a storage of the enzyme in a form which is not readily dispersed in aqueous solution.

In the 31-day-old plant, the cotyledons, which are about to fall off, still contain approximately 80 per cent. of the total urease of the plant. The marked decrease in urease content between the 15-day-old and 31-day-old plant is due primarily to the rapid decrease in urease of the cotyledons themselves. Even after the cotyledons have fallen off, however, the total urease of the plant continues to decrease.

A composite picture of the distribution of urease in the various parts of the developing seedlings of soy bean 8, 15, 31, and 52 days after planting is represented in figures 1 and 2. The stippling indicates the relative urease content as revealed by the histological methods. The numbers opposite each

plant part are the number of urease units per gram of fresh weight of that plant part as determined by the quantitative method.

Discussion

Comparison of urease distribution in soy bean and jack bean

It was shown in the second paper of this series (**11**) that, in all the organs and plant structures examined, the urease changes follow the same course. The urease activity of an organ per gram of fresh weight (curve A, fig. 5)

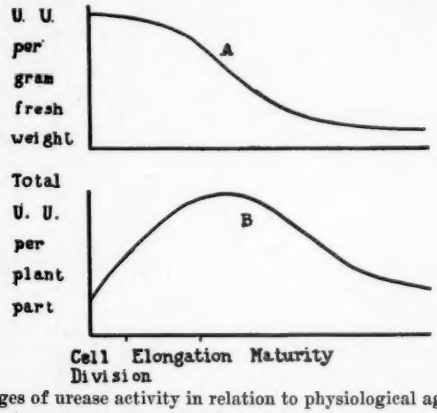

FIG. 5. Changes of urease activity in relation to physiological age. Curve A, change of urease activity, of an organ per gram of fresh weight, with age; curve B, change of total urease content of an organ with age.

is highest when the cells are still meristematic and decreases as the cells grow older. The total urease content of an organ (curve B, fig. 5) increases rapidly during the period of cell division until, at about the time primary growth has ceased, the organ contains a maximum urease content. From that time on, there is a decrease in urease content which is more or less marked, depending on the individual organ considered. It was further shown that the changes in urease activity noted in the organs are due primarily to changes taking place in the parenchyma cells of the organs since neither the cambium nor its derivatives contain detectable amounts of the enzyme. The total urease activity of the meristematic cell increases rapidly at first (curve B, fig. 5); and as the cell passes into the stage of elongation the rate of increase of urease activity drops off. About the time the cell has acquired its maximum size it contains a maximum amount of urease. After this time there is a gradual decline of urease activity per cell until some constant low level is reached. This series of changes found in *Canavalia ensiformis* is likewise found in *Soja max*.

There were, however, some minor differences. In general the urease concentration was less in soy bean than in jack bean. The palisade cells of the soy bean cotyledon contained somewhat more urease than did the other parenchyma cells; and in the 8-day-old seedling, urease was present in somewhat greater concentration at the periphery of the soy bean cotyledons than toward the inner region. In the jack bean cotyledon no such differences in distribution were observed. There was a more rapid decrease in urease activity in the pith than in the cortex of the soy bean hypocotyl during germination. In the jack bean, however, the decrease in the hypocotyl was somewhat more rapid in the cortex than in the pith.

Urease in other legumes

That the curve of urease changes is not merely limited to the two plants investigated but holds also for other legumes is indicated by the experiments of Kiesel and Troitzki (**12**). Their limited data on the urease changes in a number of legume plants are in accord with the general changes of urease in the jack bean and soy bean plants. For example, the young developing seeds of *Phaseolus vulgaris* and *Pisum sativum* contained one-fifth the urease per gram of fresh weight of the older seeds. The leaves of a *Pisum* seedling three weeks old contained more urease per gram of fresh weight than did the stems. Cotyledons of 10-day-old seedlings of *Vicia faba* had about five times the urease activity per gram fresh weight that cotyledons of 28-day-old seedlings had. The changes in urease distribution and concentration found in the soy bean and jack bean may then be considered as changes which are at least common to the Papilionaceae.

A comparison of distribution of urease and other enzymes

Burk (**5**) has called attention to enzymic activities which are growth-bound, *i.e.*, "where the enzymic activity is correlated with the structure of the living cell to the extent that the velocity of formation of the (intracellular) reaction product parallels and is normally measured by the velocity of growth. Such growth-bound enzymes may be termed phyo-enzymes."

Since the enzyme urease appears to be so intimately bound up with the growth of the parenchyma cell, one may consider urease as a growth-bound enzyme. It was interesting to see whether other hydrolytic enzymes might likewise be growth-bound and undergo changes similar to those found for urease. In table IX data are presented on the analyses of urease, protease, and lipase in the developing seed. The urease content increases markedly during the period of seed ripening. Tovarnitzky's analyses (**25**) of the nitrogenous fractions of soy beans at different stages of development show that the increase of urease activity parallels the increase in the soluble protein content and this investigator calls attention to the fact that "A reliable

TABLE IX

ANALYSES OF SOY BEAN SEEDS AT DIFFERENT STAGES OF DEVELOPMENT

ANALYSES	SIZE OF BEANS IN MM.									
	3.5–5	5–6	6–7	7–9	10–10.5	11.5–12.5	13.5*	13.5†	12 YELLOW BEANS DRYING	9 YELLOW BEANS DRY
Amino-N in mg.										
Per gm. fresh wt.	2.94	2.84	2.88	2.92	2.34	2.16	.....	.....	.....	5.72
Per gm. dry wt.	12.0	17.1	18.9	13.4	9.87	6.85	.....	.....	.....	7.10
Per bean	0.0311	0.0750	0.0960	0.195	0.376	0.600	.....	.....	.....	1.09
Free NH_3 in mg.										
Per gm. fresh wt.	0.156	0.145	0.156	0.124	0.130	0.184	0.192	0.135	0.155	0.124
Per gm. dry wt.	0.588	0.871	1.02	0.568	0.549	0.583	0.522	.....	0.301	0.154
Per bean	0.00165	0.00382	0.00519	0.00282	0.0209	0.0512	0.0766	0.0545	0.0485	0.0239
Urease activity in mg. NH_3 produced per min.										
U.U. per gm. fresh wt.	0.010	0.0164	0.0139	0.0555	0.0596	0.243	0.497	1.20	2.21	2.92
U.U. per gm. dry wt.	0.037	0.0986	0.0891	0.254	0.252	0.770	1.35	.....	4.28	3.65
U.U. per bean	0.00010	0.00043	0.00048	0.00370	0.00958	0.0675	0.199	0.485	0.691	0.558
Protease activity in mg. NH_2-N produced in 48 hr.										
Per gm. fresh wt.	12.85	11.00	11.85	10.60	10.00	7.30	.....	.....	.....	9.40
Per gm. dry wt.	48.4	66.1	77.7	48.6	42.2	23.2	.....	.....	.....	11.7
Per bean	0.136	0.290	0.395	0.708	1.61	2.03	.....	.....	.....	1.80
Lipase activity in cc. of 0.1 N acid produced in 48 hr.										
Per gm. fresh wt.	3.36	.....	3.40	2.74	3.86	4.68	.....	.....	.....	1.12
Per gm. dry wt.	12.6	.....	22.3	12.6	16.3	14.8	.....	.....	.....	1.39
Per bean	0.0356	.....	0.113	0.183	0.621	1.30	.....	.....	.....	0.214
Titrable acidity in cc. of 0.1 N acid										
Per gm. fresh wt.	0.432	.....	0.381	0.341	0.352	0.203	.....	.....	.....	0.284
Fresh wt. per bean	0.0106	0.0264	0.0333	0.0668	0.161	0.278	0.400	0.404	0.313	0.191
Dry wt. per bean	0.00281	0.00439	0.00507	0.0146	0.0382	0.0876	0.147	.....	0.161	0.154
Percentage water in bean	73.5	83.3	84.8	78.1	76.2	68.7	63.3	.....	48.6	19.4

* Hilum light pink; pod turning yellow.

† Hilum dark purple; pods dark yellow; beans yellow green.

index for the amount of protein in soy beans, its solubility and the maturity of the beans, is the activity of urease." As the bean matures, urease accumulates in the cells, some of it being stored in the form of ergastic amorphous substances just as other globulins are stored. The water content in the later stages is decreasing, the cellular materials becoming more and more concentrated; and the semi-fluid vacuoles of the cells take on the appearance of solid spherical corpuscles. Although the storage phase is evident at the last stage (*i.e.*, a decrease of urease activity in the 9 mm. yellow bean) it is highly probable that some storage of the protein urease has occurred even in the earlier stages of seed development.

This increase of urease activity as the seed matures is not paralleled by an increase of protease or lipase activity.[4] It is seen from table IX that the protease and lipase activities per gram of dry weight of seed increase at first, reach a maximum activity in the 6- to 7-mm. bean and then decrease as the bean enlarges. It is interesting to note that the values for amino nitrogen and free ammonia per gram dry weight, and for the percentage of water content are also highest in the 6- to 7-mm. bean. These results on protease and lipase activities of the developing bean do not agree with those of RIVKIND and TOVARNITZKY (**25**) who report a slight increase of these enzymes in the maturing seed. These investigators made analyses on the air-dried seeds, and do not describe their methods of analyses. The determinations reported in table IX were made on freshly picked material without preliminary drying; corrections were made for the varying values of autolysis, which become important in estimating the very low activities of both protease and lipase found in the soy bean. In this connection it may be mentioned that, in the developing seeds of *Vicia faba*, BLAGOWESTSCHENSKI (**3**) found that the amylase content per gram dry weight of seed decreased as the seeds ripened.

Upon germination of the seeds the aleurone grains and crystalloids absorb water and are seen gradually to take on a semi-fluid appearance. In the beans grown at 27° C. (table I) the values for urease activity per gram of fresh weight of the cotyledon increased up to a maximum value on the second day and then began to decrease. [The germinated beans, used for the determinations on protease and lipase (table X), were not those related to the developing beans of table IX, so that no direct comparison can be made between the protease and lipase activities of the developing and germinating beans.]

[4] Proteinase activity was measured by permitting aliquots of fresh tissue mash to act on a 2 per cent. casein solution for 48 hours at 30° C. in the presence of phosphate buffer (pH 6.5) and overlayered with toluene. Amino-N was determined by the Van Slyke nitrous acid method. Lipase activity was measured by the increase in titratable acidity of a gum arabic-castor oil emulsion, containing about 2 per cent. fat, when acted upon by 0.5 per cent. tissue mash for 48 hours at 30° C. and overlayered with toluene. (Toluene does not interfere with the determination.) Titration to the phenolphthalein end-point is made on an aliquot of an alcohol-ether extract of this mixture.

* Hilum light pink; pod turning yellow.
† Hilum dark purple; pods dark yellow; beans yellow green.

The protease activity per gram of fresh weight of the cotyledon reached a maximum in two days and then slowly decreased. From the complete hydrolysis of proteins there will be produced some 7 mg. NH_2-N per bean. This indicates that protein digestion during germination is significantly limited by the low protease activity. Lipase activity increased in two days and was apparently highest in the 11-day-old stage; it then rapidly decreased. In a microchemical examination of the germinating soy bean

TABLE X

ANALYSES OF COTYLEDONS OF GERMINATING SOY BEANS

	Days after planting						
	1	2	5	8	11	15	30
Fresh wt. per cotyledon pair	0.274	0.286	0.590	0.512	0.496	.....	0.264
NH_2-N in mg. per gm. fresh wt.	2.32	2.70	2.98	3.40	1.68	0.861	.....
Protease activity in mg. NH_2-N produced in 48 hr. per gm. fresh wt.	3.30	4.25	.....	2.70	1.45	0.27	0.078
Lipase activity in cc. of 0.1 N acid produced in 48 hr. per gm. fresh wt.	1.48	3.41	2.24	2.39	4.58	0.86	0.00

VON OHLEN (**26**) reports that in the cotyledons a marked decrease of oil had occurred by the fourth day; depletion began at the base of the cotyledons and progressed toward the opposite end; the palisade layer was depleted last. The acidity produced by the complete hydrolysis of the fat per bean is equivalent to 1 cc. of 0.1 N acid. The small amount of lipase present and the fact that the fat decreased markedly by the fourth day indicate that the rate of fat hydrolysis was limited greatly by the amount of active lipase present. On germination urease and protease increase during the first two days and then decrease, while lipase attains a maximum activity on the eleventh day and then decreases rapidly.

The data of FISCHER (**9**) indicate that the proteases of peas, beans, and buckwheat increase in the leaves with age. This differs from the changes of urease found in the leaves examined by the writer in which there is a rapid increase in urease to maturity and then a decrease. In the root tip urease is highest in concentration in the region of cell division and decreases rapidly in the elongation region. Peptidase of the barley root tip, however, is highest (**18**) in concentration in the region of cell elongation and not in the region of cell division.

UREASE AND RESPIRATION

If urease, unlike protease and lipase, is a growth-bound enzyme it might be expected that the changes in respiratory activity would normally parallel

the changes in urease activity, and that both of these factors would parallel, and be a measure of growth changes. A comparison of the data on the respiratory activity of legumes, as found in the literature, with the data on urease activity of parenchymatous tissue as here presented confirmed the suggestion that urease and respiratory activity run parallel. BEZAGU (**2**) working with leaves of various ages, of such species as *Robinia pseudoacacia, Cercis siliquastrum, Pinus sylvestris, Cobaea scandens, Ligustrum vulgare, Althaea,* and *Lovoglossum hercinum* found that the following facts were general for the respiratory activity of these leaves.

1. The amount of CO_2 produced per gram of fresh weight per hour is highest in the youngest leaves and continues to decrease with the development and aging of the leaves in a manner similar to curve A, figure 5.

2. The CO_2 production per leaf-hour is lowest for the very young leaves, increases rapidly to a maximum during elongation, and then gradually decreases as the leaf passes through the stages of maturity and senescence. These changes may be represented by a curve similar to B, figure 5.

This relation of respiratory activity to urease activity, that is, to the fact that urease is a growth-bound enzyme, is understandable upon the basis of the following assumptions:

1. Urease, in its active state, is a soluble protein which undergoes changes similar to other proteins in synthesis and decomposition.

2. The amount of soluble proteins (as distinguished from ergastic proteins), including urease, is a measure of the amount and activity of protoplasm.

3. Respiratory activity is related to protoplasmic activity or to the amount of catalytic (protein) surface upon which oxidation-reduction reactions take place.

UREASE AND PROTOPLASM

Much of the framework of protoplasm (**23**) is considered to be made up of proteins which are dispersed in an aqueous medium. The only measure of protoplasmic activity which is generally recognized is respiratory activity. Since urease forms a portion of the protoplasmic framework, it is suggested that the amount of protoplasmic framework will be proportional to the amount of active urease and to the respiratory activity. If these assumptions are correct, then the changes in protoplasmic framework or protoplasmic activity in a cell may be studied by observing the changes in urease activity under varying environmental conditions. In other words, the enzyme urease may be used as a "tracer." Although changes in respiration can be much more readily followed than urease changes in the study of protoplasmic activity, they have the disadvantage of being much more complex summation effects of numerous enzymic actions and various substrate concentrations than are the urease changes.

In the young embryonic cell there is a dense protoplasm which, as the cell elongates, later contains a number of vacuoles. With increasing age of the cell, the vacuoles combine to form a single large sap cavity so that the protoplasm becomes spread in a thin film along the cell wall. The data on respiratory and urease activity of leaf parenchyma cells are in accord, not with the interpretation that the protoplasm of the embryonic cell is merely stretched and diluted but that the total amount of protoplasm, or the protoplasmic activity per cell, actually continues to increase until the cell has attained its maximum size; after that, it decreases to a certain level as the cell ages.

Urease and physiological age

As was noted in the first paper of this series (**10**), it is only the enzyme in a dispersed state, in which its active groups are available, which can be quantitatively determined. An interpretation of the curve of urease changes with age of the cell is therefore difficult to make. It seems certain, however, that the ascending portion of curve B figure 5, illustrating the increase of urease as the cell enlarges, can be interpreted in no other way than as a synthesis of the enzyme. It has been suggested as a possibility that an enzyme might not be synthesized *in situ* but might be transported as such to the growing regions. For example, the 10,000-fold increase of urease content from the youngest to the oldest seed of soy bean might be due to a transport of urease to the developing seed. The studies on urease indicate that the enzyme is not transported as such to other cells, for the following reasons: (1) Urease has not been detected in the secondary phloem tissue. (2) The disappearance of urease from the storage parenchyma cells of a cotyledon bears no observable relation to the proximity of these cells to fibrovascular bundles in their neighborhood. (3) When jack bean cotyledons, which contain the highest recorded amounts of urease, are kept in tap or distilled water for several days, the water will give no test for the presence of urease. These results may be due to the fact that this enzyme, being a protein molecule, cannot diffuse to any extent through the plasma membrane. It is interesting to note, in this connection, Bach's report (**1**), that urease is present in *Aspergillus niger* as an endocellular enzyme and that no urease is excreted into the culture medium.

A satisfactory interpretation of the descending portion of curve B figure 5 must await a better understanding of the processes leading to senescence and death of a cell. It is difficult to evaluate the significance of the decrease in urease activity after the cell has reached the end of primary growth since the active enzyme may disappear because it is being catabolized by proteolytic ferments, or because it is being stored in a zymogen form, or because it is being inactivated. There is some evidence that catabolism of urease is

the major cause of the descending portion of the urease curve. From the work of SCHULZE and SCHÜTZ (**22**) and others it is known that leaves increase in protein N as they enlarge, and that during midsummer the content of protein N per leaf then begins to decline. This decrease in protein and in urease in the mature and aging leaves may perhaps be related to the fact that with increasing age of the leaf there is an increase in protease activity in the leaf (**9**). Peculiarly enough, the changes in urease and protease of the higher plants appear to run parallel with those found for *Aspergillus niger*. BACH (**1**) found an increase of urease in *Aspergillus*, the urease content reaching a maximum during the first 5 to 7 days and then rapidly declining to about one-sixth of the maximum value by the fifteenth day. This decline in urease content likewise coincided with an increase in proteolytic activity of the fungus. It is hoped in a future paper to deal with the question of the "element constant of protein," as related to the age and carbohydrate content of the cell, and its relation to urease activity.

RÔLE OF UREASE IN INTERMEDIARY METABOLISM

Since urease appears to be present in almost all plants and certainly to be present in all the organs of the jack bean and soy bean, what rôle does this enzyme play in the metabolism of plants? As is well known, an enzyme catalyzes the attainment of the equilibrium of a chemical reaction (**4**). Whether and to what extent hydrolysis of urea will take place will depend on the equilibrium constant of the reaction and on the presence of urea and water. The synthesis of urea will likewise depend on the equilibrium constant of the reaction and on the presence of ammonium carbamate.

It has been firmly established by MACK and VILLARS (**19**) and others that urease catalyzes the following reaction:

$$\text{urea} + H_2O \rightleftharpoons \text{ammonium carbamate.}$$

The forward reaction, that is, the hydrolysis of urea to ammonium carbamate proceeds very rapidly. It can readily be calculated[5] that about 2000 urea molecules make effective collisions per second with one urease molecule. The decrease in free energy of this hydrolysis is sufficiently great that urea is almost completely transformed into ammonium carbamate.

That urea can be absorbed by a legume plant from a sterile culture medium and can be split very rapidly into ammonia by the urease present in the cells has been reported by KLEIN (**13**). He has also shown that if large amounts of urea are fed, the ammonia produced becomes so great that signs of ammonia poisoning appear and the plant turns brown and dies. If one stops the feeding of urea, the total urea taken up disappears in a very short time.

[5] Assuming a molecular weight of 150,000 for crystalline urease; an activity of 26,000 U.U. per gram of crystalline urease (**24**); one active spot per urease molecule; excess of substrate; and that the reverse reaction is negligible.

The question of the presence in the plant of the substrate, urea, may be considered briefly. The investigations of KLEIN and TAUBÖCK (**14**) indicate that little free urea is present in the higher plants. The "urea" reported by previous workers was a complex of ureides which are apparently widely distributed throughout the plant kingdom. The ureides of both the higher plants and the fungi are split by boiling with dilute acetic acid; boiling with dilute alcohol does not decompose them. The ureides of higher plants are readily soluble in alcohol; the ureides of fungi are not. They report only two ureides in higher plants: the formaldehyde compound, which is found only in green assimilating plants; and the acetaldehyde compound which is formed in green unlighted or in chlorophyll-free organs as a result of respiratory processes. FOSSE believes that urea is present in combination with glyoxylic acid as allantoic acid. These stable ureide bodies remain in the organs for some time but at certain definite periods are attacked and disappear. Since the enzyme urease is present in the plant, it may be concluded that any free urea, released from the ureide combinations, from arginine, canavanine, purines, guanidine, etc., will be rapidly decomposed to liberate ammonia. The presence of large quantities of urease in the meristematic regions of the jack bean and soy bean suggests that urease performs a "necessary" function in releasing ammonia for protein synthesis.

Concerning the synthesis of urea by urease it has been postulated by ONSLOW (**21**), EULER (**7**), and others, that urea might be formed by the action of urease on ammonium carbamate. It has been realized, however, that when urea is brought into contact with urease an almost complete conversion of urea to ammonia results. But there remained the possibility that synthesis could occur in the cell if the cell had some means of removing from the field of enzyme action the small amounts of urea formed. For example, the trace of urea might be used to form a ureide, and then more urea would be produced because of the equilibrium involved.

It is possible to arrive at some definite conclusions as to the significance of urease in the synthesis of urea by making use of the data of LEWIS and BURROWS (**16**) on the hydrolysis of urea, and on the conversion of ammonium cyanate to urea (17). If urea, ammonium cyanate, ammonium carbamate, ammonium carbonate, or their ionic products are brought into water, an equilibrium will finally be established in which all of these substances will be present in certain definite proportions. For example, it may be calculated, that starting with a 1 M. urea solution in a closed container at 25° C. the products at equilibrium will be approximately 0.998 M. ammonium carbamate-carbonate, 0.002 M. urea, and a trace of cyanate.

Likewise, the maximum concentration of urea that may be synthesized by urease may be calculated. The highest amount of ammonia nitrogen in the jack bean, equivalent to 0.1 M. NH_3, was found in the radicle of a

16-day-old seedling (**14**). Assuming that sufficient CO_2 is present to combine with all the ammonia present, then at this ammonia concentration approximately 80 per cent. of the ammonia will be in the form of ammonium carbonate and the other 20 per cent. will be in the form of ammonium carbamate, in a slightly alkaline medium (**8**). If this plant tissue is assumed to be neutral, the maximum theoretical amount of urea at equilibrium with this concentration of ammonium carbamate will be 1.8×10^{-6} gm. per gram of tissue. Even though the urea thus formed is slight, it might be removed from the sphere of urease action possibly to form a ureide and thus permit further urea synthesis.

Ammonium carbamate is, however, unstable on the acid side of neutrality and is very rapidly converted to carbonate. No ammonium carbamate is formed from ammonium carbonate in plants because plant tissues have a hydrogen-ion concentration that is generally on the acid side of neutrality. Since the enzyme urease catalyzes the reaction of ammonium carbamate to urea and water, no urea could be synthesized because no ammonium carbamate would be present; and no ureides would be formed. It may therefore be concluded that urease in the plant does not function in the synthesis of urea.

From the data on free energy change it becomes evident that no matter what steps are postulated for the synthesis of urea from ammonia and carbonic acid as they exist in the plant, some energy source, such as a coupled oxidation, is required (**15**). It is interesting to note that asparagine, another storage molecule for ammonia, is, like urea, an amide and also requires a coupled oxidation process to supply the energy for its synthesis (**20**). Whether urea formation by synthesis from ammonia is to be considered an important factor in explaining the significant quantities of ureides present in plants remains to be seen. The investigations of FOSSE suggested to KREBS (**15**) that "urea formation in plants follows mainly the same path as urea formation from purines in amphibians" that is, through the hydrolytic formation of urea from ureides rather than through a direct synthesis from ammonia.

The interesting paper by FEARON[6] ought to be mentioned. FEARON advances the hypothesis that cyanic acid is in some way responsible for urea formation. He believes that carbamic acid may form cyanic acid which together with ammonia will produce urea.

Insufficient attention has been paid by many investigators of hydrolytic enzymes to the energy values and equilibria involved in hydrolysis. LEWIS and BURROWS (**16**) established the fact that the free energy value in aqueous solution at room temperature of ammonium cyanate is greater than that of

[6] FEARON, W. R. The structure of urea with reference to its deamination and synthesis by urease. Biochem. Jour. **30**: 1652–1660. 1936.

urea; likewise the free energy value of urea is greater than that of ammonium carbamate; *i.e.*, ammonium cyanate → urea → ammonium carbamate. If one assumes a 1 M. urea solution at room temperature and that no decomposition takes place to ammonium carbamate, it can be calculated that at equilibrium there will be 0.0067 M. ammonium cyanate and 0.9933 M. urea. But since urea is decomposing to ammonium carbamate, at equilibrium little or no cyanate will be present.

Any hypothesis which assumes that carbamate produces cyanate which in turn produces urea must in addition postulate an energy source for the synthesis of cyanate from carbamate.

Summary

1. The urease activity of *Soja max* has been determined by the use of histological and quantitative methods. The data for total urease are summarized in the graph (fig. 4), which shows the total urease content of the developing bean, and of the soy bean seedling throughout the life history of the plant. The distribution of urease in the various tissues of seedlings 8, 15, 31, and 52 days old is illustrated in a composite picture (figs. 1, 2). A description of the specific urease changes of the individual plant structures is given.

2. In the soy bean, as in the jack bean, the changes observed in urease content of the plant are due primarily to the changes in urease content of the parenchyma cells. Neither the cambium nor the cells derived from the cambium contain amounts of urease that can be detected with the indicator reagents.

3. The changes in urease distribution found in the jack bean and soy bean may be considered to hold for the Papilionaceae in general.

4. Analyses of urease, lipase, and protease in the developing soy bean indicate that the changes in lipase and protease activities do not follow the changes in urease activity.

5. Urease is considered a growth-bound enzyme. There is a rapid synthesis of urease in actively dividing cells. Synthesis continues during the stage of cell elongation, at the end of which period the urease content of the cell reaches a maximum. The urease content of the cell then begins to decrease to a certain level.

6. The changes in respiratory activity and urease activity at various stages of cell development follow similar courses.

7. It is suggested that by observing the changes of urease activity one may obtain an indication of the changes in the protoplasmic framework and soluble protein content of a cell.

8. The data on urease and respiratory activity are in accord with the interpretation that protoplasmic activity in the parenchyma cell continues

to increase until the cell has attained its maximum size; after that it decreases to a certain level as the cell becomes older.

9. The rôle of urease in the intermediary metabolism of the plant is discussed. From thermodynamic considerations it is calculated that any urea present in the plant cell will be converted by urease into ammonium carbamate. Ammonium carbamate, being unstable on the acid side of neutrality (the condition in the plant cell), will be hydrolyzed to ammonium carbonate. It is also shown that urease cannot function in the synthesis of urea from ammonium carbonate in the plant cell. The presence of large quantities of urease, especially in the meristematic or protein synthesizing regions of the jack bean and soy bean, suggests that urease performs the function of releasing ammonia from any free urea present and thus makes it available for protein synthesis.

10. There is no evidence that urease is transported, as such, from one portion of a plant to another.

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SPECIFIC FACTORS OTHER THAN AUXIN AFFECTING GROWTH AND ROOT FORMATION

F. W. WENT

(WITH SIX FIGURES)

Introduction

One of the most startling discoveries in the field of plant growth hormones is that auxin affects not only the growth in length of stems, petioles, etc., but also inhibits lateral buds, induces root formation, swellings, cambial activity, etc. This led to the conception that the auxins were stimulating substances without a clearly defined physiological activity or specificity (FITTING, 5). In a recent paper (WENT, 27) it has been pointed out that all effects of auxin other than cell elongation must be called secondary and that they all can be accounted for if we assume that other specific substances are activated in one way or another by auxin. A few experimental results pointing toward such an interpretation were offered but in the following paper the experimental data are given in full. It was endeavored to show that only in combination with other specific growth factors auxin influences root formation, bud growth, or swellings. It also gives an example of a new type of experimentation which has become possible through the availability of crystalline auxins. Whenever a plant or a part of a plant is flooded with auxin, other factors necessary for growth and development will become limiting, and in this way the existence of such factors can be demonstrated, and their concentration measured. The effects of the unphysiologically high auxin concentrations, however, must not be taken to mean that the naturally occurring auxin acts in the same way in the normal plant, as has been done recently (HITCHCOCK and ZIMMERMAN, 9). In the following paper auxin is interpreted only as a reactant, not as a factor in normal development.

The auxin used in all experiments to be described is indole-3-acetic acid, the so-called "hetero-auxin." The peas are a pure line of Alaska obtained through the courtesy of Dr. W. BROTHERTON. All experiments are carried out in a darkroom with controlled temperature (24° C.) and humidity (85 per cent). In darkness growth is simpler to interpret because synthesis of a number of substances essential or accessory for growth will not take place, and we need to consider only those already present or synthesized in darkness.

Stem and bud growth

If we cut off the cotyledons from peas germinating in darkness, when the epicotyl has reached a length of one or a few centimeters, while the roots are

left in water, the growth of the shoot stops very soon. This is due to a lack of nutrients, because if a 2 per cent. sucrose solution is offered to the cut surface left by the removal of the cotyledons, growth is resumed and continues for a considerable time, at least for one week. Addition of auxin to the sugar solution has no effect. The same effect is produced when the roots are immersed in the sugar solution. This proves that the uptake of the sugar, necessary for growth, can take place either through the roots or through a cut surface. At least the roots are not necessary for the sugar uptake.

These results seemed, however, to contradict those obtained in the experiments on root formation, in which a piece of stem with an apical bud was cut off at the base and placed in sugar solution. The growing region of the stem in this case practically stopped elongating. From a number of other experiments, however, it appeared that for this growth in length of the stem *both* sugar and the presence of roots are necessary. To substantiate this statement two experiments are summarized in table I. Peas are germinated on wet filter paper in the darkroom. As soon as the shoots are about 1 cm. long they are transferred to small bottles, one per bottle, into which a few cc. of water are poured. When the plants have reached a suitable length (about 10 cm.) they are divided into four equal groups. One group is left intact, with both roots and cotyledons; in the second group the roots are cut off just below the cotyledons, which are left; in the third group the cotyledons are cut off close to the stem, leaving the roots; and in the fourth group the roots with cotyledons are cut off just above the insertion of the latter. In the first and second group the seed coats are removed to prevent premature decay. Then each bottle is filled with 5 cc. of 2 per cent. pure sucrose.

The growth of the four groups is summarized in table I; the results are clear-cut and are borne out by a number of other experiments not recorded here. In the first experiment the growth has been recorded for the first four days after the operation only: the growth of the plants without either roots or cotyledons is intermediate between those with or without both. In both groups lacking roots the growth drops off much more rapidly in the third and fourth day than in the groups with roots. The same is seen in the second experiment, where the growth has been followed for nine days: in the last period of four days the growth of the plants without cotyledons but with roots is even twice that of those without roots but with cotyledons, so that the former actually overtook the latter in length. This second experiment is also shown in figure 1, where the relatively constant growth rate of both groups with roots stands out.

When both roots and cotyledons are cut off the shoots practically cease growth. Only during the first two days is there a small residual growth.

TABLE I

TOTAL GROWTH AND GROWTH RATE OF ETIOLATED SHOOTS OF PEA SEEDLINGS AFTER COTYLEDONS OR ROOTS OR BOTH HAVE BEEN CUT OFF. LENGTH OF SHOOTS AT BEGINNING OF EXPERIMENT 10–15 CM. EACH FIGURE IS THE MEAN OF 10 PLANTS

CONDITION OF PLANTS	EXPERIMENT 1			EXPERIMENT 2			
	GROWTH IN 4 DAYS	GROWTH RATE IN MM./DAY FOR PERIOD		GROWTH IN 9 DAYS	GROWTH RATE IN MM./DAY FOR PERIOD		
		1–2	3–4		1–2	3–5	6–9
	mm.	*mm./day*	*mm./day*	*mm.*	*mm./day*	*mm./day*	*mm./day*
With cotyledons with roots	101	26.5	24.1	206.5	32.0	29.7	13.3
With " without roots	48	15.1	9.0	80.8	16.9	10.9	3.6
Without " with roots	38	10.0	9.0	91.9	15.2	10.9	7.2
Without " without roots	8	3.4	0.7	19.0	5.2	1.4	1.1

The most outstanding fact seems to be that plants with cotyledons, but without roots, having an abundant food and sugar supply, are far behind in their growth compared with normal plants. This result is not changed if the plants are grown under a bell jar in an atmosphere saturated with water vapor, indicating that the absence of the roots does not primarily interfere with the water uptake. Whereas in the course of a week the growth of the groups without roots but with cotyledons, and those without cotyledons but with roots, has reached about the same total, the type of growth is completely

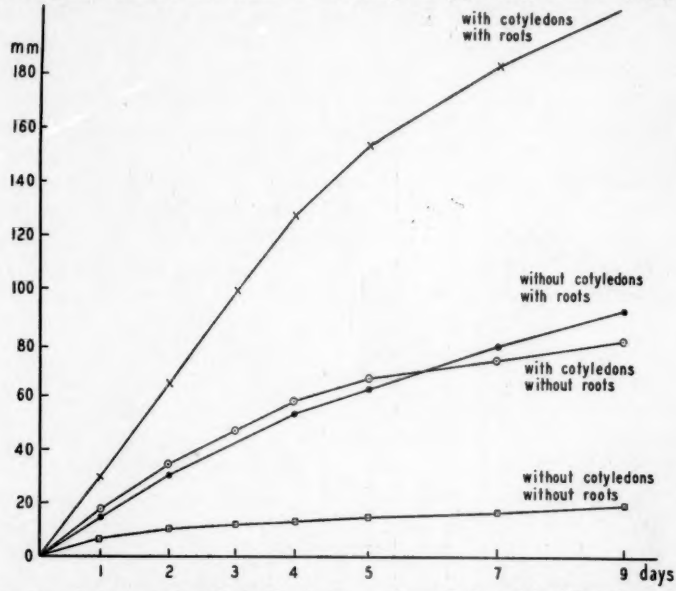

FIG. 1. Increase in length (ordinate in mm. beyond the original length of 100–150 mm.) of etiolated pea seedlings, after removal of roots or cotyledons. Abscissa: days after operation. Each line represents the mean of 10 plants.

different. In the first case the growth drops off rather rapidly, indicating a rapid depletion of the factor necessary for growth in length, whereas in the second case it is evident that this factor is continuously supplied. Thus we reach the conclusion that the roots form, and the cotyledons store to some extent, a factor required for growth in length of the stems. There is no doubt about the necessity of auxin for the elongation of the stem, so that the factor coming from the roots has to be considered as a second growth factor, which has to cooperate with auxin to make growth possible. A lack of either will stop stem elongation. It is very likely that this factor is one of those summarized under the name "food factor" (WENT, **23, 25**).

A question arises about the nature of this factor coming from the roots. It might be that the roots are able to concentrate sugar and whatever salts may be present in the sugar solution. This is not likely, because as shown in the beginning of this section the effect of the roots is apparent only when sugar is present, and this sugar may be taken up through the cut cotyledonary petiole.

To obtain further evidence a number of grafting experiments have been performed. The same etiolated pea seedlings are used. When the stem has reached a length of about 10 cm. it is cut off just below the first node. Then

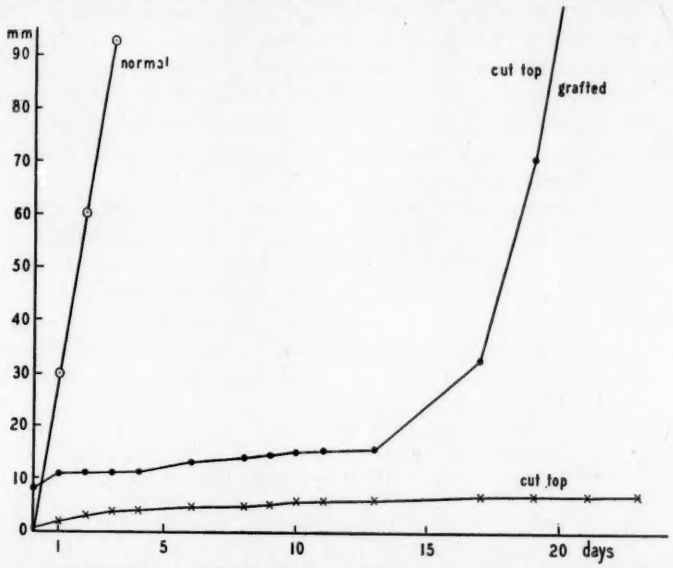

FIG. 2. Increase in length (ordinate represents mm. growth beyond the original length of 100–150 mm.) of etiolated pea seedling stems, in the course of 23 days after operation (abscissa). Circles: growth of normal untreated plants. Crosses: growth of shoots cut above the cotyledons. Black dots: growth of cut shoots, grafted on their roots + cotyledons.

it is replaced, sealed with gelatin or agar, and held in place by a short piece of glass tubing with a bore of the same diameter as the stem. Although the gelatin eventually becomes liquified by bacterial activity it still gives distinctly better results than agar. Recently still better results have been obtained by direct contact of the tissues without the use of gelatin or agar. The growth of the grafted stems is then measured for a period exceeding ten days. During the first two days some residual growth still takes place just as in the experiments in which cut stems are placed in sugar solution.

Growth then ceases completely, as will be seen from figure 2. After one or two weeks a certain number of the grafted stems suddenly resume growth and develop normally, at practically the same rate as not-operated seedlings. It has been found that in all those cases where growth is resumed a fusion of the tissues of graft and stock has occurred, and that this fusion is still lacking in the others which do not grow. The fusion takes place between the vascular tissues. This experiment, which has often been repeated, shows that the factor, coming from the roots and necessary for the growth of the shoots, does not pass a cut surface but moves through living tissue only. Whether this is due to inactivation of the active substance outside the cells or otherwise, has not been determined as yet. For the argument, however, this is not essential. Thus these grafting experiments confirm the conclusion, that a special substance is formed in the roots, which moves upwards from cell to cell towards the apical parts of the pea stems where it causes growth in length of the shoot in conjunction with auxin. I propose to call this substance *caulocaline*.

This factor coming from the roots is the same as the factor necessary for growth of the axillary buds, as shown by the following experiments. If the same four groups of peas are made: one with both cotyledons and roots, one with cotyledons but without roots, one with roots but without cotyledons, and one without either roots or cotyledons, and if these plants are then placed in 2 per cent. sugar solution and decapitated, only the first three groups will develop axillary buds, as shown in table II. In these experiments the effect of the roots is still more pronounced: with roots but without cotyledons the growth is almost twice that of the plants without roots but with cotyledons. This difference from the experiments on the growth of the main shoots (table I) is easily explained and even to be expected, for we have seen already that the effect of the cotyledons is greatest in the first days,

TABLE II

GROWTH OF AXILLARY BUDS IN PEA PLANTS DECAPITATED IMMEDIATELY AFTER REMOVAL OF ROOTS OR COTYLEDONS. EACH FIGURE IS THE MEAN OF 10 PLANTS

CONDITION OF PLANTS	EXPERIMENT 1		EXPERIMENT 2	
	GROWTH OF AXILLARY BUDS		GROWTH OF AXILLARY BUDS	
	WITH AUXIN	WITHOUT AUXIN	WITH AUXIN	WITHOUT AUXIN
With cotyledons with roots	7.1	65.8	8.3	26.3
With " without roots	3.6	14.0	1.0	1.9
Without " with roots	5.4	22.7	3.9	21.2
Without " without roots	1.0	1.0	1.0	1.0

and then drops off rapidly because of a depletion of the caulocaline-factor in the cotyledons. In the plants with roots there is a continuous supply of this factor, even after a week. The axillary buds start to grow rapidly only four or more days after decapitation, when the plants with cotyledons but without roots are low in the growth factor coming from the roots. Table II also offers a good example of bud inhibition by auxin. In the columns "with auxin" the plants are treated immediately after decapitation with a concentrated auxin paste. This delays the bud growth, but does not otherwise change the results. This must be interpreted to mean that auxin inhibits lateral buds *through* this other factor necessary for bud growth, caulocaline.

Another remarkable and consistent effect has been noticed in all these experiments. In the plants with cotyledons but without roots, just as in those with both, only the buds in the axil of the upper scale develop, whereas in the plants without cotyledons but with roots the buds on the lower node or the cotyledonary buds (which are not removed with the cotyledons) grow out. The explanation has to be sought in the relative concentrations of auxin and caulocaline. In all cases the buds present start to grow out, but soon the lower or the upper overtakes and then inhibits the others. With an abundant auxin supply (when cotyledons are present) the upper bud inhibits the lower through its auxin production. Without much auxin (cotyledon cut) the upper buds do not inhibit the lower ones; as the latter are closer to the caulocaline supply they will grow better.

Swellings

The growth of stems stops soon after decapitation because of lack of auxin. Growth stops after cutting the stem at the base because caulocaline becomes limiting. Addition of auxin to the apical cut end of a decapitated pea stem has an effect on elongation only in the lowest concentrations. Slightly higher auxin concentrations (in contrast with the *Avena* coleoptile) inhibit growth in length and still more auxin causes the upper few mm. of the decapitated stem to swell. Thus there is a continuous series of concentrations giving first elongation, then growth inhibition and swellings. The following experiments make it probable that not only for growth in length but also for swellings the cooperation with auxin of a factor coming from the roots is necessary.

Again the same four series of plants are prepared and placed in sugar solution. They are decapitated and a concentrated auxin paste (1 part indole-3-acetic acid in 500 parts lanolin) is applied. In another series the decapitation is carried out two or three days after removal of the roots or cotyledons. At that time the plants should have used up whatever growth factors they had at the moment of de-rooting, and only in those plants with a continuous supply should swellings occur. Table III summarizes the re-

TABLE III

DIAMETER OF APICAL SWELLINGS OF PEA STEMS DECAPITATED 5 MM. BELOW TIP AND TREATED WITH CONCENTRATED AUXIN PASTE. DECAPITATION AND APPLICATION OF AUXIN EITHER IMMEDIATELY AFTER CUTTING OF COTYLEDONS AND ROOTS, OR TWO DAYS LATER. EACH FIGURE IS THE MEAN OF 10 PLANTS

CONDITION OF PLANTS	EXPERIMENT 1		EXPERIMENT 2	
	SWELLINGS WHEN		SWELLINGS WHEN	
	AUXIN ON IMMEDIATELY	AUXIN ON AFTER 2 DAYS	AUXIN ON IMMEDIATELY	AUXIN ON AFTER 2 DAYS
	mm.	*mm.*	*mm.*	*mm.*
With cotyledons with roots	4.4	4.2	5.5	4.6
With " without roots	4.0	2.8	5.0	2.5
Without " with roots	4.4	3.6	4.0	3.6
Without " without roots	3.7	2.4	3.6	2.4
Stem diameter before treatment	2.2	2.2	2.2	2.2

sults of two such experiments. It will be seen that auxin by itself is not able to produce appreciable swellings. Only in cooperation with something coming from the roots do the swellings appear. Again this factor moves through living tissue only, as evidenced by the following experiment. Many pea stems are cut off and sealed on their respective root systems (with cotyledons) again. At set intervals the length of the shoots is measured, and some of them are decapitated and strong auxin paste is applied. Only in those plants in which the growth of the shoots has been resumed (indicating the successful junction of the tissues) appreciable auxin swellings appear. Although the direct experiment still stands out there is strong evidence that the same factor coming from the roots is necessary to give growth by cell elongation—in conjunction with low auxin concentrations—or will give swellings with excess auxin. The probable relation between such swelling and growth in length has been discussed elsewhere (WENT, **27**).

CZAJA (**4**) has observed that when the cotyledons are removed from *Vicia faba* seedlings no swellings occur after auxin treatment. From the picture he publishes it is clear, however, that his effect may have been the result of a lack of roots rather than lack of cotyledons, as he concludes. Based on their own experiments JOST and REISS (1936) conclude that removal of cotyledons does not prevent the formation of auxin swellings on pea epicotyls. Since we know that auxin effects (like bud inhibition) may be transmitted across a cut surface even without actual junction of the tissues of the graft (SNOW, **19**), the experiments mentioned above with grafted pea stems form another argument against the hypothesis of CZAJA (**3**) that the swellings occur as the result of two streams of auxin, one coming from the base.

Leaf growth

It still might be argued that in all the preceding cases the roots contain a certain amount of food necessary for growth and development in general, or that they are able to transform sugar into such food, so that every growth

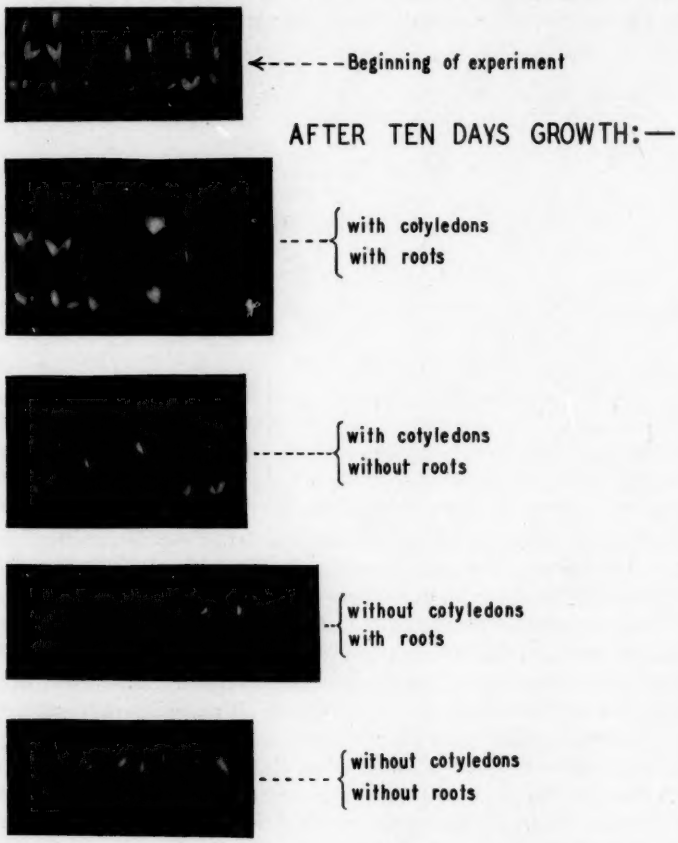

FIG. 3. Shadow photographs of all leaves with petioles and stipules of etiolated pea plants in one experiment. Leaves + terminal bud of each plant are cut off and arranged in a vertical row. Above, leaves of a set of plants at the moment the cotyledons or roots were cut off the others.

process will be speeded up by the presence of roots. This is by no means the case. The effect of the roots is of a more special nature. For if we inspect the plants used for the experiments summarized in table I, a very marked

difference will be observed in the size of the terminal buds, leaves, and stipules, which differences do not go parallel with the presence or absence of roots. Figure 3 shows shadow pictures of all the leaves which have developed on the different groups of plants, and also the size of the leaves on a set of plants just before treatment, which can be considered as the controls. Table IV contains the actual measurements taken from these pictures.

TABLE IV

MEASUREMENTS OF LEAF DIMENSIONS OF THE EXPERIMENT FROM FIGURE 3 TEN DAYS AFTER REMOVAL OF EITHER COTYLEDONS OR ROOTS

CONDITION OF PLANTS	MEAN LEAF SURFACE			PETIOLE AND LEAF LENGTH				
	1ST LEAF	2ND LEAF	TOTAL	1ST	2ND	3RD	4TH	TOTAL
	*mm.*²	*mm.*²	*mm.*²	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
With cotyledons with roots	17.7	24.5	42	12.8	14.3	10.2	4.6	41.9
With cotyledons without roots	22.0	19.5	41	13.2	11.3	6.6	3.1	34.2
Without cotyledons with roots	14.3	±10	24	9.8	8.5	5.2	2	25.5
Without cotyledons without roots	13.8	±10	24	10.3	8.4	4.6	2	25.3
Before treatment	13.8	±10	24	9.4	8.3	5.0	2	24.7

Where the cotyledons are cut off neither leaves nor stipules increase in size, even in presence of the roots. On the other hand, absence of the roots does not prevent the leaves from developing, provided the cotyledons are left, in which case they almost double their leaf surface in 10 days. The same relationship is found if we measure the length of leaf blade plus petiole: no increase in length occurs if the cotyledons are absent. This result has been confirmed in another experiment; indeed, in all experiments reported here this phenomenon is met with. It accounts for the curious shape of the stems growing in sugar solution with their cotyledons cut off. During normal growth the terminal bud continues to increase in size by the successive development and growth of new leaves, so that when a new internode elongates and the leaf at its lower node does not envelop the bud any more, this bud has grown and has the same appearance as the bud in the younger plant. But when the cotyledons are cut, neither the terminal bud nor its enveloping leaves grow any more, so that when through growth of the stem the successive internodes develop and peel off the preformed leaf primordia from the terminal bud, the latter seems to shrink until it is not more than an insignificant appendage, and such a stem looks almost pointed. The same thing happens with the growth of the axillary buds: when only roots are present

they develop into long spear-like shoots, whereas the lateral buds on plants with cotyledons are topped by a fair-sized bud.

If etiolated pea plants, even without cotyledons, are placed in light of sufficient intensity, the stipules and leaf blades will develop and grow considerably. This necessity of light for leaf blade growth is well known, and the lack of leaf development in darkness is one of the characteristics of the phenomenon of etiolation. In KOSTYTCHEW-WENT (**11**) a good example of the effect of light of even short duration on the growth of *Vicia faba* leaves is depicted (p. 294). GREGORY (**6**) has quantitatively measured the increment in leaf surface of *Cucumis* plants under controlled conditions, and concludes that a special factor, necessary for leaf growth, is formed in the older leaves under the influence of light. This factor is not directly related with photosynthesis of carbohydrates, since the Q_{10} of photosynthesis is very different from that of leaf growth.

I observed a curious case of deficient leaf growth in extreme shade in the Botanic Gardens in Buitenzorg, Java. *Gonocaryum* grows there in the shade as a small tree. Owing to its large thick leaves it casts a dense shade on the ground so that only a few of the most pronounced ombrophilous plants can grow there. The fruits of this tree are large and enclose only one seed, which contains an excessive amount of storage food. The germination of a fruit, lying under the tree, is normal, and a shoot with many normal leaves develops. The shade, however, is so dense that soon these leaves are shed and the growth of the main stem stops. Axillary buds then develop, the first of these shoots still bearing some leaves, although their leaf blades are small and mottled. Apparently the reserves of leaf-growth substances in the seed are exhausted long before the rest of the storage food and the auxin is used up. Thus for many months such seedlings continue to send up shoots, without leaves, but with very small scales which develop instead. If such a seedling with the appearance of a witches'-broom, and without a single leaf, is brought into full daylight, then at once new, though small, normal leaves start to develop instead of scales. This observation seems also to warrant the conclusion that in the light new leaf growth substances are formed.

The differentiation between various growth factors by removal of cotyledons, roots, apical buds, etc., is relatively rough; a much more elegant way is afforded by the use of monochromatic light. This at least is indicated by experiments which have been performed by growing cut pea shoots in different colored lights, obtained by filtering the light of a strong incandescent lamp through sets of Corning glass filters. Yellow and blue light seemed to favor leaf growth; red favors stipule development; bud growth was best in orange and yellow. Green light was practically ineffective for all processes studied; plants subjected to it behaved as if they were grown in darkness.

In the case of leaf-blade growth practically no evidence exists which links the activity of leaf-growth substances with auxin. Quite an amount of circumstantial evidence, however, points toward the conclusion that auxin specifically conditions petiole and vein growth in the leaf, that is, conditions growth of those tissues which are built up from the beginning by elongated cells. Experiments, described by LAIBACH (**12**) and AVERY (**1**), show that auxin after one-sided application causes bending of the midrib due to a growth increase on the side of the application. I have confirmed these experiments on a number of different plants and have reached the conclusion that auxin does not seem to increase mesophyll growth, although it increases vein growth. The same effect, but in opposite direction, has been described by MARESQUELLE (**16**). When leaves have been attacked by lice, the mesophyll bulges out without being affected otherwise, because only the growth of veins and midrib has been decreased as a result of the sucking of the aphids. Similarly there are some virus diseases known which specifically affect stem and vein growth, and in one such case GRIEVE (1936) has shown that this is correlated with a lack of auxin in the virus-attacked plant. In all these cases only vein growth is affected, and not the mesophyll. The opposite case: deficient mesophyll development without change in vein growth is found in certain hereditary deficiencies, "deformis" in tobacco (HONING, **10**) and "wiry" in tomatoes (LESLEY and LESLEY, **15**). In view of this indirect evidence it seems sufficiently established that the growth of a leaf is a complex phenomenon as far as the growth of its constituents is concerned. Auxin regulates midrib and vein growth, but does not influence mesophyll development, which is governed by a special leaf growth substance formed in leaves in the light and stored in pea cotyledons, which I propose to call *phyllocaline*.

Root formation

In the preceding sections we have seen that apart from auxin another factor is needed for growth in length of pea stems, and that, when these stems have been cut off, this factor disappears from them within about two days. A similar (or identical) factor, necessary for the production of swellings by excess auxin, likewise disappears in the course of two days after cutting the stems.

In the case of root formation a slightly different situation might be expected, because to induce the initiation of roots we deliberately cut the pea stems near the base before we treat them with auxin. This might be interpreted by assuming that auxin by itself is sufficient to induce root formation. That this is not true will be shown in the following section.

In previous experiments on root formation the number of roots, visible to the naked eye near the basal cut surface of a pea stem fourteen days after

treatment, has been used as a measure for the effectiveness of the hormone treatment (WENT, **24**). When treated with relatively low auxin concentrations in water, under the prescribed conditions, the number of roots formed is rather small and the number of visible roots approaches the total number initiated. In the present studies, however, excessively high auxin concentrations have been used (0.5–2 mg. of indole-3-acetic acid per gram of lanolin), and since the lanolin is left on the treated plants, the auxin action is continuous over a long period of time. Under these conditions only a small number (20 to 40 per cent.) of all roots initiated by the treatment grow out and become macroscopically visible. This is mainly due to two factors: in the first place, an excess of auxin prevents root elongation, and secondly, the material necessary for root growth in length is limited, so that an increase in number of roots initiated will cause a decrease in their ultimate size, until the majority never grow out. This means that, in the experiments to be described, the roots had to be counted under a microscope with the aid of cross sections of the treated pea stems. Since it was found that the middle part of such stems never form any root primordia, the upper and lower 5 mm. of each stem (or a longer region if necessary) have to be cut by hand into sections of about 0.1 to 0.2 mm. thickness, which are placed in order on slides. With a low power the root primordia are then easily visible at the transition between central cylinder and cortex, mostly alternating with the four primary vascular bundles of the central cylinder, or in connection with the four small cortical bundles. Care has to be taken not to count the same root twice if it appears in consecutive sections. Very close to the point of auxin application at the apical cut surface the root primordia occasionally seem to have fused into a wide meristematic plate, and then arbitrarily this has been (mentally) divided into a certain number of root primordia.

The first experiment to be described is directly comparable with the previous experiments on growth in length. Etiolated pea seedlings are divided into three groups: from one the roots are cut off, from another only the cotyledons are removed, and the plants from the third group are left intact. Each plant is placed in a separate bottle with a few cc. of 2 per cent. sucrose solution. After four, six, or seven days a few of the treated shoots are cut off just above the second scale and placed in 2 per cent. sucrose; the 5-mm. tip is removed and a concentrated auxin paste then applied to the apical cut surface. Seven days later the peas are sectioned and the number of roots initiated is determined. Table V summarizes the results. For each stem individually is recorded the number of roots formed near the apical and basal cut surface of the pea stems. The figures marked "total" give the total number of roots formed per plant per treatment. It seems that as the plants grow older root formation on stems drops somewhat. This

TABLE V

NUMBER OF MICROSCOPICALLY VISIBLE ROOTS AND ROOT PRIMORDIA FORMED ON CUT PEA STEMS. THEY HAVE BEEN CUT AND TREATED AT THEIR APEX WITH AUXIN PASTE 4, 6, OR 7 DAYS AFTER REMOVAL OF EITHER COTYLEDONS OR ROOTS

STEMS CUT FROM PLANTS	ROOTS FORMED	TIME BETWEEN REMOVAL OF ROOTS OR COTYLEDONS AND AUXIN APPLICATION					
		4 DAYS		6 DAYS		7 DAYS	
With cotyledons with roots	At apex	20		13		9	
	At base	8		7		7	
	Total		28		20		16
With cotyledons without roots	At apex	21		8		6	
	At base	8		4		5	
	Total		29		12		11
Without cotyledons with roots	At apex	10, 9, 3		2, 3		1, 2	
	At base	3, 3, 3		1, 0		2, 0	
	Total		10		3		2.5

decrease is only about half the original value of that in the presence of the cotyledons, but when the cotyledons have been removed root formation practically falls to zero. There is a great difference between the group with cotyledons but without roots, with a total average of 17 roots formed per plant, and the group with roots but without cotyledons with an average of only 5 roots. Both groups had about the same growth in length, so that here we have a clear case of two processes, both dependent upon auxin, which are independent of each other because of the intervention of other factors: root formation is conditioned by auxin and a factor supplied by the cotyledons, and growth in length takes place under the influence of both auxin and caulocaline.

The experiment described above makes it clear at once that root formation is not merely a function of the cell as such, even if it is supplied with sufficient auxin, but it is rather a function of the specific condition of the tissue, depending upon the presence of a specific factor coming from the cotyledons which cooperates with auxin. This conclusion is confirmed by the following set of three experiments. In each of them a number of long (30 to 40 cm.) etiolated pea shoots, grown under standard conditions, are selected for uniformity. These shoots are cut off just above the cotyledons and 5-mm. tips are removed. They are then either left intact or cut into 2, 3, 4, 6, 8, or 10 pieces. Each piece is placed with its base in 2 per cent. sugar solution, and all sections from one plant are either treated by applying auxin paste (1 part indole-3-acetic acid in 500 parts of lanolin) to the apical cut surface, or are not treated and left as controls. Nine days later both the

apical and basal portions of each piece are sectioned, and in figure 4 the actual number of roots counted in each case is listed. The vertical lines represent the pea stems; where these lines are interrupted the stems have been cut. Each number from 1 to 24 (top row) represents one stem; all figures below one stem number refer to roots formed on the sections of that stem. All numbers at the right of the upper part of a section are the roots formed

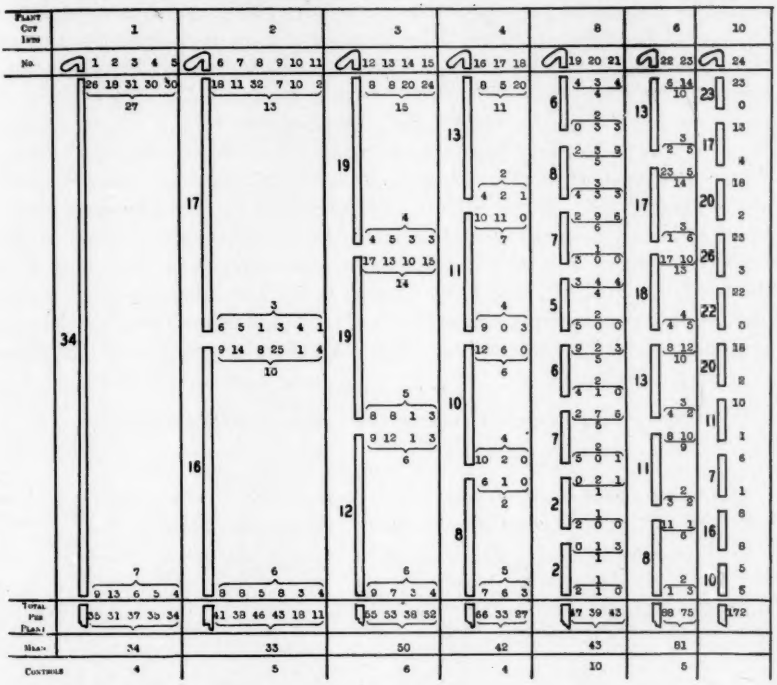

FIG. 4. Root formation after apical auxin application, at apical and basal cut surface of sections of pea stems. The stems have prior to auxin treatment been cut into 1, 2, 3, 4, 6, 8, or 10 sections. To the right of each section are the number of roots formed at apex and base in the individual plants, and the means. To the left is the total mean.

near the apical cut surface; to the right of the lower end of each section are indicated the roots formed near the base. For convenience sake the means and totals of roots formed are calculated, per apical or basal end as well as per section (to the left of each line representing the section) and per plant (bottom row). Plant numbers 5, 10, 11, 17, 18, 20, and 21 belong to the first experiment; 1, 2, 6, 7, 12, 13, 16, and 19 to the second; and 3, 4, 8, 9, 14, 15, 22, 23, and 24 to the third. It will be seen that there is an enormous variation in the root formation per apex or base, although the totals per plant are

more constant. This variation is only partially due to the fact that the results of three individual experiments have been combined.

If we try to draw a conclusion from figure 4, then it is clear that one point stands out. If for a moment we disregard the plants 22, 23, and 24, it is clear that the number of roots formed per plant is the same irrespective of whether such a stem has been cut into 1, 2, 3, 4, or 8 pieces. This is not caused by a constant root formation at the apex of the apical section and at the base of the basal section, but the number of roots formed at the apex of the top section is roughly inversely proportional to the number of sections cut from the stem, indicating that the roots are formed at the expense of some factor distributed over the whole length of the stem. For this factor the name rhizocaline may be suggested, previously used for the root-forming factor moving downward in the stem which was found to be identical with auxin (WENT, **26**). The maximal root formation on one stem only occurs when an excess of auxin is applied at the tip of each section. Similar sections, not treated with auxin, form only a few roots, and these only near the basal cut surface. The latter appear under the influence of the auxin present in the plant, and almost exclusively on sections bearing either a scale or a leaf with axillary bud. Table VI gives a summary of the influence of scale or leaf (or perhaps rather of their axillary buds) on root formation in sections treated or not treated with auxin. It shows that everywhere there is enough rhizocaline present to form a considerable number of roots after auxin treatment, independent of the presence or absence of buds, scales, or leaves. The number of roots formed in the controls decreases from the more basal towards the more apical nodes which has to be interpreted in terms of a decreasing auxin production from basal to apical lateral buds. THIMANN and SKOOG (**21**) found the opposite in *Vicia faba*, but their experiments were carried out with plants grown in the light. The presence of buds has in itself no influence on the distribution of roots over apical and basal cut surface of a section after auxin treatment. The ratio of roots at apex to roots at base is 3.5 : 1 when a leaf, 3.5 : 1 when a bud, and 4.0 : 1 when no node is present in the section.

TABLE VI

ROOT FORMATION ON PEA STEM SECTIONS FROM LONG ETIOLATED PLANTS

ROOTS FORMED PER SECTION	SECTION COMPRISING				
	1ST SCALE	2ND SCALE	1ST LEAF	2ND LEAF	WITHOUT NODE
Controls, no auxin	4.0	2.3	1.1	0.8	0.0
Treated with auxin ...	5.5	8.0	9.6	14.8	10.1

Perhaps the most interesting conclusion, with far-reaching implications, to be drawn from figure 4 is the following. From the root formation on the 4th or 8th sections of the stem it is evident that the rhizocaline is present everywhere in the stem, but that its concentration drops towards the base of the stem. Now the root formation on the non-cut stems shows that all rhizocaline present in them becomes active at either tip or base, even that portion which was present in the middle sections of the stem. This leads to the conclusion that auxin causes a redistribution of the rhizocaline in the stem.

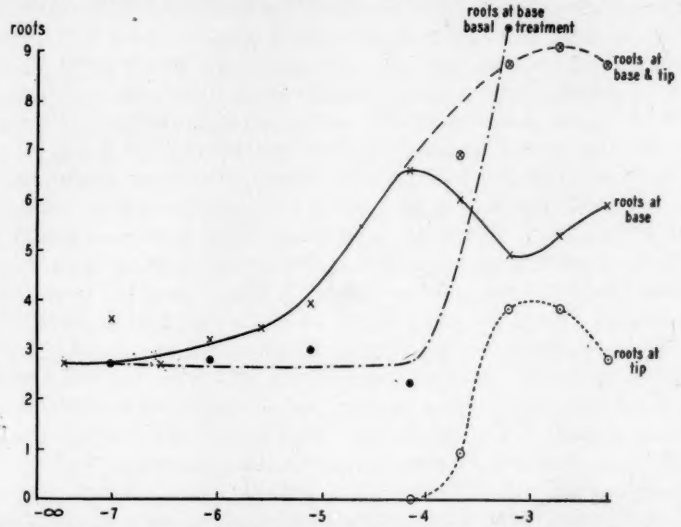

FIG. 5. Macroscopically visible roots (ordinate: roots formed per plant) on pea stems, 14 days after apical (circles and crosses) or basal (black dots) auxin treatment. Abscissa: log of the auxin concentration (in mol per liter).

The next experiments confirm this conclusion. Before considering them, however, we may examine for a moment the plants 22, 23, and 24 of figure 4, which show such a different behavior. Plants from the same series cut into 1, 2, and 3 sections formed a total of 36, 44, and 45 roots, behaving like the others in that the number of sections does not materially influence the total number of roots formed per stem. But those cut into 6 and 10 sections have formed 81 and 172 roots. It is impossible to give any satisfactory explanation for these exceptions to the scheme adopted; that we have to consider them as exceptions also follows from the following experiments.

Three hundred pea shoots, all about 15 cm. long, are cut above the first scale and 5 mm. below the terminal bud in the same way as plants are prepared in the standard test for root formation (WENT, **24**). They are treated

for 14 hours with auxin solutions in water of varying concentration, either at the base or at the (split) tip. For six days they are left standing with their base in 2 per cent. sucrose solution, then transferred to tap water, and 14 days after the beginning of the experiment the number of macroscopically visible roots is counted, both at the tip and base. The results are reproduced in figure 5 (partly redrawn from WENT, **27**). It is evident that after apical auxin treatment the number of roots formed near the base increases with increasing auxin concentration. Around 10^{-4} molar concentration the maximal number of roots is formed; beyond that concentration it decreases again. At the same time, however, roots are formed near the apex; if the numbers of roots formed near base and apex are added together the curve representing the relation between auxin concentration and root formation rises smoothly towards a maximum of 9 roots per plant. Below 10^{-4} molar the root formation caused by indole-3-acetic acid is perfectly polar, as in the normal plant. But above that concentration the polarity seems partly reversed. Exactly the same happens when the plants have been treated with auxin at their base. Below 10^{-4} molar auxin basal treatment has no effect whatsoever on root formation, but immediately above this concentration the maximal number of roots (9.4 per plant) is formed near the base. At that concentration (10^{-3}) the polar uptake of auxin seems to be abolished. A still higher concentration is highly toxic and thus does not cause any root formation at all. To compare this behavior with what happens inside the normal plant, we have to bear in mind that the auxin concentrations occurring in nature are of the order of 10^{-6} molar or less. This means that in the normal plant, and also in plants treated with auxin concentrations up to 100 times normal, root formation is a perfectly polar phenomenon: apical auxin application induces root formation at the base, basal application does not have any effect at all. Auxin concentrations of 10^{-4} molar or higher induce root formation at the place of application.

That the experiment of figure 5 is by no means an exception is shown by a number of earlier experiments which will not be considered in detail. In three experiments, each comprising about 200 plants, it was found that in order to cause root formation on pea stems by basal auxin treatment a 100–1000 times higher auxin concentration was required than for apical treatment. Numerous experiments have shown that when very concentrated auxin solutions are applied to the tip of pea cuttings, the number of roots formed at the base is less than with lower concentrations, and simultaneously roots appear near the treated tip. These roots are able to grow out, because the high auxin concentration acts only during the 15 hours of application, and the growth of the roots occurs later when the excess auxin has disappeared.

It is evident from these experiments that the roots at the apex are formed at the expense of those at the base. Here again we have a very clear

case of a developmental process being limited by a factor other than auxin, and we reach the same conclusion: namely, that a factor, which we will call rhizocaline, is present in pea stems in limited amounts. Without this factor no root formation is possible. Low auxin concentrations inside the stem make the rhizocaline move downwards—in the same direction as the polar auxin movement—and cause root formation at the base. At high auxin concentrations, above 10^{-4} molar, roots will form at the place of auxin application, independent of the innate polarity of the stem.

Discussion

If we survey the evidence collected in the foregoing experiments there are a few important points which should be stressed and considered in their relations to other known facts.

Four different processes in the development of a pea seedling have been studied: namely, growth in length of stems and lateral buds, apical auxin swellings, root formation, and leaf growth. The first three were known to depend on the presence of auxin, and since up to the present it has been principally the effects of auxin on these processes which have been studied the specificity of auxin has consequently been questioned. Thus the old idea of stimulus—which never had quite disappeared from the minds of the older generation of plant physiologists in relation to development—came up again. Thus, auxin should "stimulate" cells, and, according to the disposition of each cell, it should grow in length, swell, form roots, or do something else. In another paper (WENT, **27**) this problem has been discussed in greater detail. Here it suffices to say again that the stimulus concept involves a setting free, or release, of the energy of a cell previously in a metastable equilibrium. This involves no stoichiometrical relationship between stimulus and reaction. Now one of the most striking properties of the auxins is their quantitative action; the more we come to know about them, the clearer this stands out. For a whole series of compounds (including indole-acetic, indole-propionic, indole-butyric, indole-valeric, naphthalene-acetic and anthracene-acetic acid) it has been found that per mol they all cause the same amount of growth, provided all other conditions are strictly comparable. That means that the influence of auxin on growth can only be explained on the basis of a chemical master reaction leading to growth, in which the molecules of the auxin take a part, and not by a stimulation "releasing" the "growth energy."

The other part of the comparison between auxin and stimulus may seem more to the point. Auxin does not cause only growth in length, but it also affects other cell activities. If *only* auxin were involved in these cell activities, then auxin might be considered to set free one reaction or the other, that is to say, to stimulate, the cells determining what they were going to do once

auxin were there. But even in the writer's first paper on the growth hormone (WENT, **23**) it was pointed out that in order to explain the auxin effect on growth it was necessary to assume a second factor, the food factor ("Zellstreckungsmaterial"). The experimental evidence for the presence of such a food factor was decidedly insufficient, but the theoretical foundation was sound. Later (WENT, **25**) better experimental evidence for the food factor has been collected, so that a theoretical discussion of the relation of auxin to growth should include consideration of such a food factor. Recently LAIBACH (1936) arrived at similar conclusions; his second factor is identical with either the food factor or the auxin precursor.

Independently of any previous theoretical considerations it has been shown in this paper that it is not the presence of auxin which determines whether elongation, swelling, or root formation will take place in a pea stem, but rather more specific, independent factors, which have been named "calines." Their existence so far has not been directly proven, but enough evidence has been collected to make their existence highly probable. In addition the assumption of the presence and action of such calines offers the simplest explanation of many other effects related to growth and development of plants. A plant with cotyledons cut, that is, low in caulocaline but high in rhizocaline, will not grow in length or form swellings when auxin is applied, but will form roots in abundance; conversely, it is possible to get plants low in rhizocaline but high in caulocaline. They will form swellings without roots when concentrated auxin paste is applied to their apical cut surface. Also phyllocaline and caulocaline can be independently affected in the pea stem, causing good stem elongation without leaf growth, the reverse, both, or none. The possibility for the demonstration of these factors lies, like that for auxin, in their hormonal nature. They are formed or stored in one part of the seedling and are effective at another place. They disappear during their action, so that a constant supply is needed. Fortunately the different calines are formed or stored at different places in the plant. Caulocaline is formed in the roots, and the cotyledons have only a small storage, which becomes depleted in about one week after the removal of the roots. The stem contains an amount hardly sufficient for one day's growth. Phyllocaline is stored in the cotyledons, is formed in leaves in light, and is not present in the stem to any extent. As soon as the cotyledons are cut off, leaf growth stops. Rhizocaline also comes from the cotyledons, but it is present in the stem in considerable quantities, which only disappear gradually (in the course of 4 to 6 days when the roots are left on the stem, but the cotyledons removed). In a diagram representing a pea seedling (fig. 6) the place of formation of the various hormones is indicated.

A connection of auxin to phyllocaline is not clear or perhaps even nonexistent, but, to make caulocaline or rhizocaline effective, the presence of

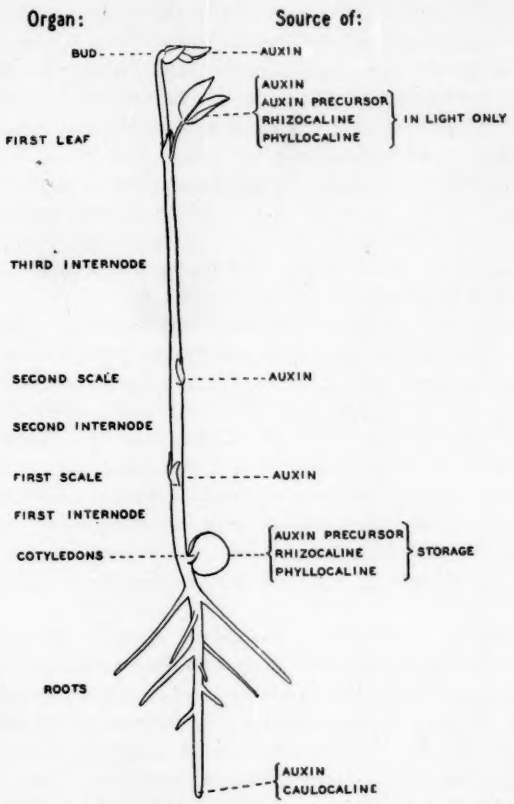

FIG. 6. Pea seedling with indication of places of formation and storage of various hormones discussed in the present paper.

auxin is required. The mutual relation between rhizocaline and auxin is the best investigated; hence it may be considered first. Without auxin no root formation is possible. If roots are formed without the specific addition of auxin it means that the plant contains some auxin already. But besides the necessity of the mere presence of auxin to render rhizocaline active, it affects rhizocaline in still another way. Auxin in suitable concentrations conditions almost quantitative accumulation of rhizocaline either near the base or near the apex of a cut stem. It is clear that it is not the auxin concentration in itself which is responsible for this accumulation, since no roots are formed in the middle portions of the stem, where, especially after treatment with high auxin concentrations, a high auxin content should be ex-

pected. It rather seems to be the auxin gradient which causes movement of rhizocaline. The naturally occurring polar auxin movement will cause an auxin accumulation near the base of a stem. If a very high auxin concentration is applied to the apex, then a second auxin gradient may be set up, especially when the auxin transport mechanism becomes inadequate to handle the large excess of auxin at the tip. How much rhizocaline will accumulate near apex or base, *i.e.*, the proportion of the total number of roots which will be formed at apex or base then depends upon relative magnitudes of the apical and basal gradients. Basal auxin treatment only will be effective when the concentration is high enough to cause basal uptake; it then will increase the normal gradient.

There is only one recent paper (COOPER, **2**) which has a direct bearing on our subject. In it COOPER has shown that treatment of lemon cuttings with a high auxin concentration at the base results in an abundant root formation especially if the leaves are present. If, after the first auxin application, the treated portion of the stem is cut off and the cutting is treated again with auxin under the same conditions, no root formation beyond that of untreated cuttings results. This phenomenon COOPER explains on the same basis as used in this paper: the auxin causes an accumulation, near the base, of a second factor, rhizocaline, necessary for root formation. The rest of the cutting is then depleted in this factor, and retreatment does not have an effect.

Having seen how auxin affects the distribution of one of the calines in the plant, the same mechanism for the caulocaline may be examined. Lateral buds grow only when the apical bud is removed, and when enough caulocaline is available. Through the investigations of THIMANN and SKOOG (**21, 22**) and SKOOG and THIMANN (**18**) we know that the effect of the apical bud is exerted through its auxin. As long as it produces auxin the stem just below it grows, which means that it is supplied with caulocaline. If we now assume that auxin causes the caulocaline to move upwards in the stem and to accumulate near the place of auxin production, the dual effect of auxin on growth in length of stems, *viz.*, promotion of stem growth and inhibition of lateral bud growth no longer seem mysterious. As long as the caulocaline moves towards the apical bud, the lateral buds are not able to grow. But as soon as the apical bud stops forming auxin, the lateral buds with their slight auxin production are able to divert the caulocaline and grow. When the auxin production of the apical bud is supplanted by an artificial source, then also the caulocaline does not reach the lateral buds. Thus the inhibition of growth of the lateral buds is not a *direct* effect of auxin, but works through diversion of caulocaline. LAIBACH's view (**13**), that the lateral bud inhibition by auxin is due to its effect on the *growth* of other parts, has been disproven by SKOOG and THIMANN (**18**), but now the phenomenon can be ex-

plained through the effect of auxin on the *movement* of another growth *factor*. Whether accumulation of the caulocaline leads to growth or not is not important for the resultant bud inhibition.

The theory of direct inhibition through auxin can explain why an apical bud inhibits the basal buds, but it is at a loss to explain the opposite phenomenon. If, as mentioned in the section "stem and bud growth," a lower lateral bud or a cotyledonary bud overtakes the apical buds in development, then the latter are inhibited just the same. This cannot be caused by auxin, since in a plant the auxin formed moves downward only. But if the caulocaline is all used by the lower bud, the upper will be inhibited. The recent experiments of LE FANU (**14**) and SNOW (**20**) probably can find an explanation along the two-factor scheme as well.

Since the effect of roots on growth has been shown to occur through the intermediary of a hormone-like factor, which can move through the living plant, it would be very interesting to reconsider all cases in which an effect of roots on growth or development of other parts of the plant has been described. This would require a monographic treatment of the subject, which had better be postponed until more is known about caulocaline and possible other factors. A few cases might still be mentioned, if only to indicate how the facts discovered on etiolated pea stems have a much wider application and afford a better understanding of many plant growth processes.

The most marked effects of roots on stem growth have been found in the rootstock-scion relation. Such effects of one individual on another across a graft-union have generally been attributed to differences of root systems in regard to uptake of mineral nutrients from the soil (*e.g.*, WINKLER, **28**). We have seen, however, that the root system can influence the growth of the stem through a more specific hormone-like agent, caulocaline. A root system, which forms only small amounts of caulocaline, will cause stunted growth in the scion, even when the root system itself seems adequate as far as its size is concerned.

Another clear case of the effect of the presence of roots is provided by greenwood cuttings. The growth of such cuttings stops completely after a short time. It is not resumed until roots have been formed. Growth is then proportional to the extent of rooting of the cutting. Thus it is very easy to tell by the growth of the cutting whether it has rooted or not.

One of the methods to keep plants stunted or dwarf-like is to cut their roots far back or to prevent their growth otherwise (see MOLISCH, **17**), which of course will interfere with the caulocaline supply towards the stem and branches.

Remarkable and illuminating is also an analysis of the figures of HAGEMANN (**8**). He made leaf cuttings by sticking the petiole in the sand of the propagating frame and noted the time of first appearance of roots and

sprouts. He examined many hundreds of species, and found that only 32 per cent. of the species tested never formed any regenerates on their leaves or petioles; 42 per cent. formed roots only; 2 per cent. sprouts only; and 24 per cent. formed both roots and sprouts. From these figures it is at once clear that there is a close correlation between the formation of roots and sprouts; only when the former are present do sprouts also appear. But the correlation is still more pronounced. In the 142 plants which formed both sprouts and roots, not a single one had sprouts before the appearance of the roots; in 19 the sprouts appeared rather soon after or simultaneous with the roots, but in most cases it took 3 to 6 times longer for sprouts to form than for roots. This means that roots had to be present before sprouts would develop. The interpretation of these facts is evident. Leaves produce both auxin and rhizocaline. So roots can be formed on petioles, provided all other conditions are favorable. Once the roots are there, then caulocaline becomes available, and with the auxin from the leaves, sprouts are able to develop.

In the case of deciduous plants, which store not only storage food but also hormones, the necessity of leaves and roots for development will not be so apparent.

Summary

1. It has been shown that, although auxin is necessary for growth in length of stems, apical swellings, and root formation in pea seedlings, it is not the only specific growth factor involved in these processes. It could be shown that also the presence of other hormone-like factors, "calines," is required. Without caulocaline, which is formed in roots, no elongation of the stem or lateral buds takes place. Probably the same factor is necessary for the development of swellings after treatment of the apical zone of the stem with a high auxin concentration. Rhizocaline, coming from the cotyledons, must be present to cause root formation in conjunction with auxin. Phyllocaline is necessary for leaf growth.

2. Auxin causes a redistribution of the calines in the plant, and with the aid of this phenomenon polar root formation and bud inhibition can be further explained. The specificity in development, the decision as to whether under the influence of auxin roots will develop, or growth in length or thickness will take place, depends on the relative concentration of the various calines. They belong to a new group of plant hormones. So far it has been impossible to handle them outside living tissue.

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BUD REGENERATION AND ELECTRICAL POLARITIES IN *PHASEOLUS MULTIFLORUS*

W. S. Rehm

(WITH FIVE FIGURES)

Introduction

Various investigators have reported experiments on the relationship between the growth substance and the inhibition of the lateral buds in seedlings of Leguminosae. Thimann and Skoog (**24**) and Skoog and Thimann (**20**) have demonstrated that the growth hormone when applied to the cut surface of decapitated plants of *Vicia faba* reversibly inhibits the lateral buds. Hitchcock (**5**) has shown that lateral bud inhibition could be produced by the application of various chemicals, including indole-acetic acid, indole-propionic acid, or ethylene or propylene gases to decapitated tobacco plants. Thimann and Skoog (**24**) have advanced the hypothesis that the growth substance is transported directly to the buds where it prevents the buds from producing their own hormone, which, according to these investigators, prevents the growth of the buds. Laibach (**9**), on the other hand, supposes that the hormone does not act directly on the buds but produces a secondary reaction which in turn causes the inhibition. In this connection it is of interest to note that LeFanu (**10**) was unable to detect any appreciable amounts of auxin in inhibited lateral shoots of *Pisum sativum*. Boysen Jensen (**1**) obtained no release of inhibition when he applied growth hormone in various ways to the inhibited buds of several plants. Snow (**22**) has demonstrated that there is an inhibiting influence that may travel both apically and basipetally. However, in a recent paper Snow (**23**) found that the growth substance could travel upwards and cause both growth and inhibitory effects. Most of the workers in this field have found that the growth substance in physiological concentration travels only downward. Obviously the mechanism by which the growth hormone may bring about the inhibition of the lateral buds is one that has not been completely explained.

The present paper is concerned with the relationship between bioelectric currents and bud inhibition. A number of investigators have presented evidence to show that the hormone may be transported electrophoretically by the inherent potential differences. Went (**25**) has proposed, on the basis of his experiments on the uptake of dyes (which experiments have been confirmed by Clark, **4**), that the apices of these plants are negative to their bases and that this electrical polarity transports the hormone. A discussion of this theory will be postponed until later. It must be kept in mind, however,

that the longitudinal transport of the growth substance is not necessarily the only rôle that bioelectric potentials might play in these phenomena. On the basis of the present status of the work in this field, it is possible that bioelectric potentials may influence the distribution of the growth hormone in the nodes of plants with inhibited lateral buds, or they may be involved in this phenomena in some as yet undetermined manner.

In a previous paper (**17**) the writer reported that he found in intact vegetative plants of *Phaseolus multiflorus* electrical polarities of relatively constant orientation in the regions of the axillary buds. In that paper a method was described for measuring potential differences between various loci of the plant. The loci between which the potential differences were measured are labelled in figure 3A. The potential differences between A_1B_1, A_1P_1, and A_1P_1' were designated as the first node potentials, similarly, A_2B_2, A_2P_2, and A_2P_2' as the second node potentials. In "unstimulated" plants, A was found to be negative in the external circuit to the other loci at a given node and this orientation was referred to as the normal one.

A portion of the work reported here was done at the University of Texas. For the experiments done there, the same method was used as that described in the previous article. The plants were raised in a greenhouse and placed in a light chamber 24 hours before an experiment; the potentials were measured either with a potentiometer or with a Compton Quadrant Electrometer. During this last year the writer has been continuing this work as a guest of the Department of Botany of the University of Chicago. He wishes to take this opportunity to thank Professor CHARLES A. SHULL for the many courtesies extended to him. At Chicago the plants were grown and measured in a greenhouse, the potentials being measured with a Compton Quadrant Electrometer. Because of the variation of the temperature during the day, experiments involving short periods of time were performed at night in the greenhouse. Under these conditions the temperature did not usually vary more than 2° C. during any given experiment. It must be emphasized that the plants used in the experiment reported here were all definitely in the vegetative state. Essentially the same results were obtained under the conditions of the light chamber and under the conditions in the greenhouse.

Experimentation

INTERNAL POLARITIES

The first problem that the present paper is concerned with is an attempt to investigate the internal electrical polarities of the node. LUND (**11**) found, in his experiments on the Douglas fir, an internal polarity that was oriented oppositely to the external one. The possibility that the hormone

may be transported by the electrical currents makes it particularly pertinent to investigate the nature of the internal polarities.

For the purpose of measuring internal electrical polarities, small glass "pipes" of about one centimeter length were inserted into the plant so that the end of the pipe was at the center of the stem or petiole. Figure 1 shows

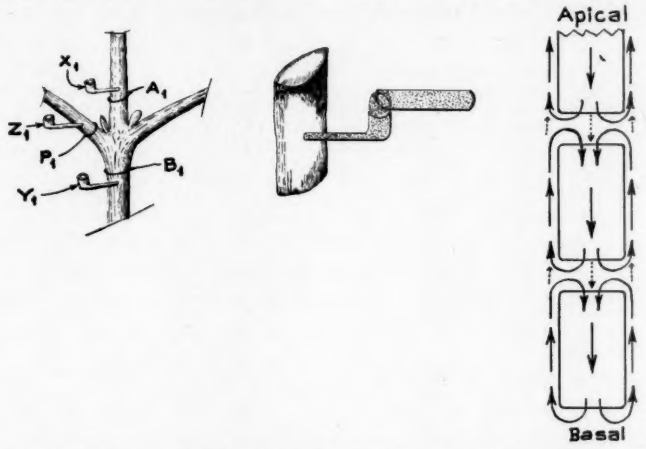

FIG. 1. Diagram on the left shows the position of the pipe contacts. The middle diagram shows the manner in which contact was made between the pipe and the contact cup. The diagram on the right represents a simplified portrayal of the possible path of current through the plant.

the method of leading off the potentials. The pipe was filled with Shive's solution and inserted into the plant. A contact cup of the same kind as shown in the previous paper (fig. 1) was filled with Shive's solution and brought close to the bowl of the pipe so that a drop of solution bridged the gap between the pipe and the contact without the contact actually touching the pipe. This arrangement permitted the plant to move without the stimulation that a rigid contact would produce. A $Zn : ZnSO_4$ electrode was placed in the contact cup and the potentials were measured in the usual way. The first measurement after placing one of these contacts in the stem showed a decided negativity of the internal contact with respect to an external contact. That is, for example, A_1 was always markedly positive in the external circuit to X_1 immediately after the pipe contact X_1 was placed in the stem. The potential difference between two such contacts decreased with time and in $\frac{1}{2}$ hour to 2 hours reached a fairly constant value. After some preliminary experiments the following definitive experiment was performed. Four intact plants were taken and contacts were placed as shown in figure 1. Since the potential difference between an outside contact and an inside contact reached

a constant level within 2 hours, it was assumed that 4 hours would allow time for the immediate effects of injury to disappear. Consequently, 4 hours after placing the contacts on and in the plant, readings were taken between the various loci at fifteen-minute intervals over a period of 4 hours. These experiments were performed in the greenhouse at night. The temperature varied from 23° C. to 24° C. The results are shown in table I. It will be

TABLE I

ORIENTATION AND MAGNITUDE OF THE INTERNAL AND EXTERNAL ELECTRICAL POLARITIES IN THE FIRST NODE. LOCI OF THE VARIOUS CONTACTS INDICATED IN FIGURE 1. POTENTIAL DIFFERENCES IN MILLIVOLTS. FOR FURTHER EXPLANATION SEE TEXT

PLANT	A_1B_1	A_1P_1	X_1Y_1	X_1Z_1	A_1X_1	B_1Y_1	P_1Z_1
	mv.	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*
1	−30	−15	−29	−29	+10	+11	− 4
2	−25	−25	−14	−14	+ 4	+15	+15
3	−25	−20	−17	−19	− 3	+ 5	− 2
4	−15	−10	−26	−15	− 1	−12	− 6

noted that the node potentials A_1B_1 and A_1P_1 possessed the normal orientation during this period. The potential differences X_1Y_1 and X_1Z_1 are all negative, that is, X_1 is negative in the external circuit to Y_1 and Z_1. This demonstrates that the internal polarities measured by this method possess the same orientation, *i.e.*, apical negativity, as do the polarities measured on the outside of the plant. The potential differences between an outside contact and the corresponding internal one are given in columns 5 and 8. The radial polarities, as can be seen from an examination of these measurements, are not as constant as the longitudinal polarities. On the basis of these results, a simplified diagram that would be consistent with both the internal and external longitudinal polarities having the same orientation is presented in figure 1.[1] This diagram represents the possible paths of current flow. It may be pointed out according to this diagram that the measured potential differences may be only a small fraction of the total RI drop in the plant. It would also follow that the total amount of current that can be drawn off from the plant may represent only a small fraction of the actual current density in certain regions within the plant. On the basis of the present experiments it is obviously impossible to say just where the cells producing the electrical potentials are located or whether or not there may be oppositely oriented gradients between the pith and the cortex. In this simple diagram no attempt has been made to represent the radial polarities.

[1] For evidence that the individual cells give rise to the measured potential differences in plants the reader is referred to the work of LUND (12), MARSH (14), and ROSENE (18).

Effect of decapitation on the node potentials

In determining the effect of decapitation on the node potentials, it is necessary to eliminate the effect of mechanical stimulation at the contacts. A sharp movement of the stem or petiole against one contact caused the stimulated region to become more negative with respect to an unstimulated region. That is, if the plant was stimulated in the region of A_1, this contact became more negative with respect to B_1; and if stimulated at B_1, B_1 became more negative to A_1. After stimulation, the potentials returned to normal in 5 to 15 minutes. To prevent this type of stimulation the plant was clamped between the node where the potentials were measured and the point of decapitation. A clamp with a large diameter was placed around the plant and the space between was filled with modelling clay; this procedure did not injure the plant in any observable way. The transmission of electrical variations produced by a mechanical stimulus has been reported for various plants by several investigators (Bose, **2**, Houwink, **6**). However, in these experiments it was found that bending the plant above the clamp or giving it a sharp blow with a glass rod produced no observable effect on the node potentials. It must be pointed out that the period of the instruments employed in the experiment reported here was 15 seconds at the minimum and that electrical variations of shorter duration than this may have escaped detection. However, these experiments in which no effect was observed in the node potentials serve as an adequate control for the following experiments on the effect of decapitation.

Decapitation of the plant produced an immediate response in the potentials of the node below the point of decapitation. In the course of various experiments, more than 30 plants were decapitated below the second node and the potentials of the first node measured. At least 5 plants were used in each of the other experiments of this group. The plants were always clamped to prevent movement against the contacts. The plants were decapitated directly above the clamp with a sharp razor. The temperature at which the various experiments were carried out varied from 20° to 28° C., though the temperature did not usually change more than one degree during the course of any one experiment.

Figure 2 shows the typical effect of decapitation below the second node on the potentials of the first node. At the time indicated by the first arrow the plant was decapitated and the node potentials greatly increased in magnitude, A_1 becoming more negative with respect to B_1, P_1, and P_1'. The magnitude of the potentials then diminished, and in about an hour they had returned to their former magnitude. The next experiments were designed to determine if this response was caused by the loss of the apex or simply by the effects of cutting. A typical response to cutting of the stem of a decapitated plant is shown in figure 2A. At the time indicated by the second arrow

a section was cut from the top of the decapitated plant. This response was similar to the first, although of smaller magnitude and duration. Even though much longer periods of time (up to 24 hours) elapsed before the second cut, this response was always of smaller magnitude than that following the original decapitation.

It was mentioned above that certain mechanical stimuli (bending and hitting) failed to elicit a response when applied above the node. However, cutting or crushing the stem of an intact plant produced a response in the

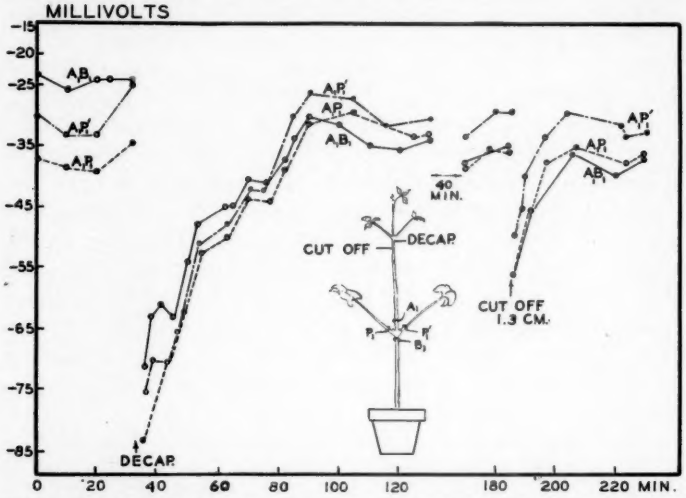

FIG. 2. The effect of decapitation on the node potentials of the first node. At the time indicated by the first arrow, the plant was decapitated. At the time indicated by the second arrow, 1.3 centimeters of the top of the decapitated plant was cut off.

node potentials. B, figure 3, shows a typical response to pressure on the stem. In this experiment the stem was squeezed with a pair of forceps with glass tubes placed over their tips. Gentle and fairly large pressures failed to produce a response in the node potentials. At the time indicated by the arrow, however, the stem was crushed by applying great pressure to the forceps. As can be seen from the figure, this caused an increase in the magnitude of the node potentials with a rather slow recovery. It was observed in plants decapitated between the second and third nodes that the buds of the first node often grew out as rapidly as those of the second node. Therefore the next step was to investigate the effect of decapitation on the first and second node potentials.

A, figure 3, shows the effect of decapitation below the third node on these potentials. The response of the second node potentials is seen to be similar

in magnitude and duration to the response of the first node potentials as represented in figure 2. In the first node potentials of the plant in A, figure 3, there was a small response in which the node potentials became slightly greater in magnitude. For convenience of presentation the response of the node potentials A_1P_1 and A_1P_1' are omitted in this figure; the response of these potentials was similar to that of A_1B_1. A similar change was found in the second node when the plant was decapitated below the fourth node. These responses of the second node below the point of decapitation were always small and sometimes were within the limits of normal variation of the

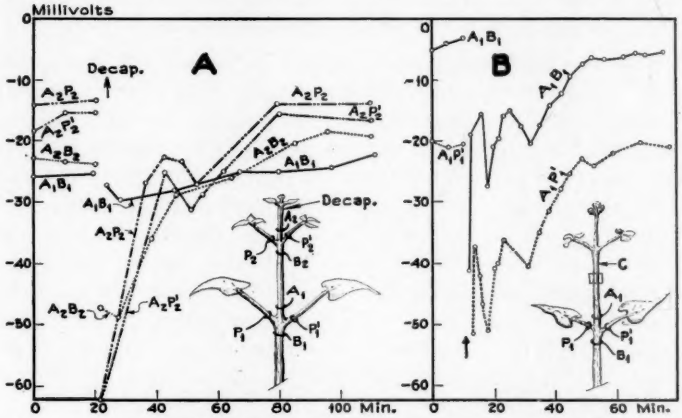

FIG. 3. A, the effect of decapitation below the third node on the node potentials of the first and second nodes. At the time indicated by the arrow, the plant was decapitated. A_1P_1 and A_1P_1' are omitted from this figure for the sake of clarity. The response of A_1P_1 and A_1P_1' was similar to that of A_1B_1.

B, shows the effect of crushing a portion of the stem on the first node potentials. A_1P_1 is omitted; its response was similar to that of A_1B_1 and A_1P_1'. The position of the clamp is shown in this figure.

node potentials. When the plant was decapitated below the fourth node, the potentials of the first node (*i.e.*, of the third node below the point of decapitation) did not change. At this juncture it may be pointed out that in 4 of these plants decapitated between the third and fourth nodes the buds of the first node grew out as rapidly as the buds of the higher nodes. In all experiments where the potentials of the node immediately below the point of decapitation were measured, a typical response, as shown in figure 2, and in A, figure 3, was observed. However, the particular shape of the recovery curves varied somewhat. The difference between the shapes of recovery curves in figure 2, and in A, figure 3, is not typical for the first and second nodes; the recovery curves of both nodes usually showed several plateaus or

dips. The experiment represented by A, figure 3, was selected in order to demonstrate that the recovery curves sometimes were suggestive of a diphasic variation. A discussion of the rôle of these responses in bud regeneration is postponed until later.

One important point to be brought out here is that, after the immediate response to decapitation, the node potentials return to approximately their former magnitude. The question then arises, is there any later response of the node potentials to decapitation, and if so, is there a relationship between it and regeneration?

Regeneration of buds and the node potentials

Procedure.—In eleven plants the node potentials and the increase in length of the buds were measured for periods of from 80 to 120 hours after decapitation. Six of these plants were measured in the light chamber described in the preceding paper, and the other 5 were measured in the greenhouse. In 3 of the plants measured in the light chamber, the potentials were measured approximately every hour over a period of 100 hours. For the rest of the 11 plants the measurements were less frequent; usually readings were made at 1- or 2-hour intervals for two 6-hour periods each day. Ten of the plants, including the 3 plants measured at 1-hour intervals, were decapitated below the second node, and the first node potentials and the increase in length of the buds of the first node were measured. One plant was decapitated below the third node, and the buds and potentials of the first and second nodes were measured.

Change in node potentials.—For convenience of presentation the results are summarized as follows. The immediate effect of decapitation was typical in all plants. The node potentials showed, after the immediate effect of decapitation was over, spontaneous fluctuations similar to those described for intact plants in the previous paper. However, it was observed after averaging the readings of each successive 6- or 10-hour interval that the average magnitude of the node potentials in each case gradually diminished after decapitation. This diminution extended over a period of from 20 to 40 hours, and in the course of this time at least one and sometimes all of the node potentials became positive. The potentials continued to fluctuate at or above zero, though in some plants they definitely returned to normal before the end of the experiment.

Figures 4 and 5 show the typical course of the node potentials after decapitation. These curves represent 2 of the 3 plants referred to above in which readings were taken at approximately 1-hour intervals throughout the experiment. B, figure 4, represents the course of these potentials in one of the plants from the time of decapitation until about 40 hours afterward. Each point corresponds to a single reading. As can be seen in this graph,

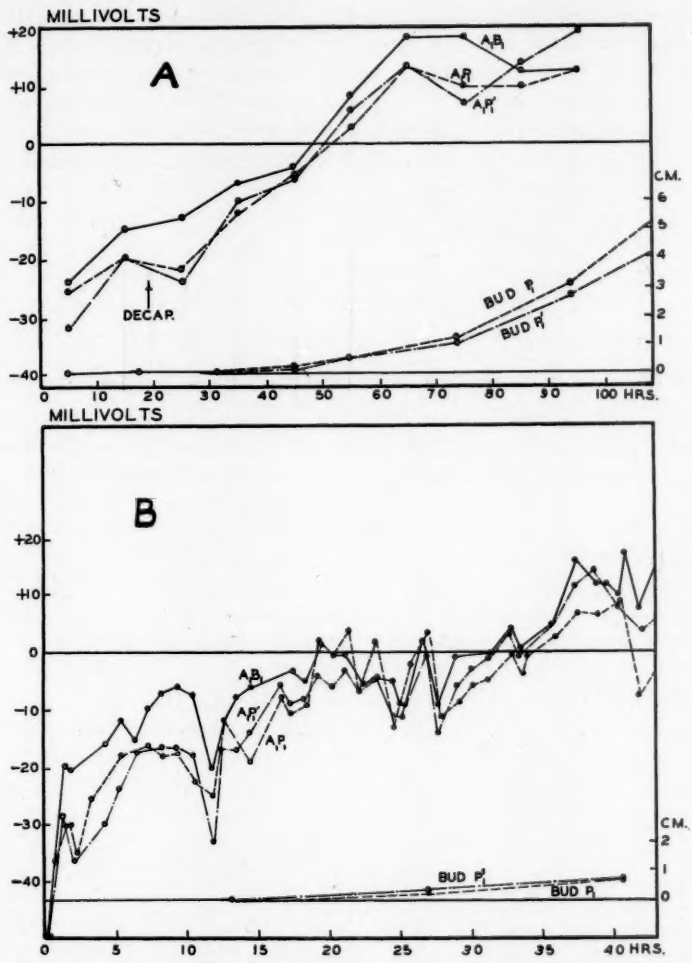

FIG. 4. Relation between the node potentials and the regeneration of the buds. A. Each point represents the average potential difference for ten hours. B. Each point represents a single reading.

these potentials fluctuate, and may return to a normal orientation for several hours, even after they have been inverted. For A, figure 4, and figure 5 the readings were averaged, each point representing a 10-hour interval. The change in the potentials shown in figure 5 is of average magnitude and illustrates the points brought out above. A, figure 4, representing the same

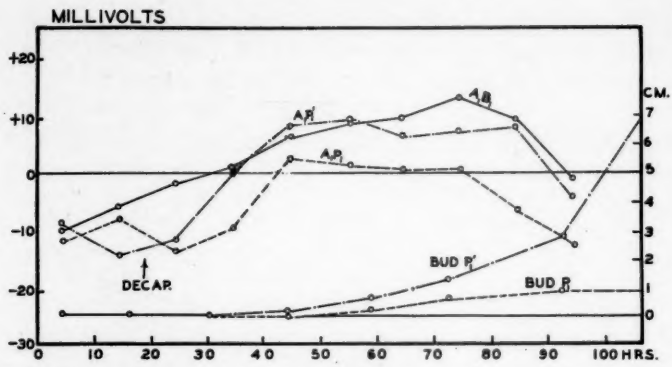

FIG. 5. Relation between node potentials and regeneration of the buds. Each point represents the average potential difference for ten hours.

plant as B, figure 4, also illustrates these points; but in this instance the change in the magnitude of the potentials was greater than usual.

TABLE II

COMPARISON OF THE AVERAGE OF THE NODE POTENTIALS IN DECAPITATED AND INTACT PLANTS. EACH NUMBER REPRESENTS THE AVERAGE OF THREE NODE POTENTIALS, A_1B_1, A_1P_1, AND A_1P_1', FOR AN INDIVIDUAL PLANT. POTENTIAL DIFFERENCES IN MILLIVOLTS. FOR FURTHER EXPLANATION SEE TEXT

PLANT	DECAPITATED PLANTS			INTACT PLANTS		
	20 HR. BEFORE DECAP.	1ST 30 HR. AFTER DECAP.	2ND 30 HR. AFTER DECAP.	1ST 20 HR.	NEXT 30 HR.	2ND 30 HR.
	mv.	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*
1	-23	- 9.6	+10	-27.5	-25	-26
2	-11	- 2	+ 8	-30	-24	-34
3	- 8	+ 3	+10	-30	-35	-15
4	-30	-15	-14	-15	-16	-18
5	-37	-26	- 8	-17	-14	-16
6	-18	-13	- 6.6	-13	-14	- 9
7	-19	- 3	+12	-12	-12	-17
8	-26	-14	- 5	-11	-22	- 8
9	-25	- 1	+ 1	-10	-16	-20
10	-15	-12	- 2	-12	-11	-15
Avg.	-21.2	- 9.3	- 0.5	-17.7	-19.4	-17.8

In table II the 3 node potentials of all 10 decapitated plants were averaged for 3 periods: (a) before decapitation, (b) the first 30 hours after decapitation, (c) and the second 30 hours after decapitation. For controls, the node potentials of 10 intact plants (from previous paper, **17**) were averaged for three periods: (a) the first 20 hours, (b) the next 30 hours, (c) and the succeeding 30 hours. In each of the decapitated plants the average

of the node potentials was more positive for the first 30 hours after decapitation than for the period before decapitation, and more positive for the second 30 hours than for the first 30 hours. On the other hand, in the intact controls the average of the node potentials was approximately the same for all three periods. As a final check on the effect of decapitation on the node potentials, the potentials of the first and second nodes of two plants raised and measured in the greenhouse were followed for 100 hours. One of these plants was decapitated below the third node, while the other one was left intact. In the intact plant, all of the node potentials remained normal throughout the entire experiment, while in the decapitated plant the potentials of both nodes exhibited the typical change after decapitation.

NODE POTENTIALS AND BUD GROWTH.—The length of the buds was measured with a pair of calipers which were adjustable by a fine screw. A mark was made with India ink on the node at the base of the bud, and the distance between the top of the mark and the tip of the bud was recorded as the length of the bud. The limit of accuracy of this method was about 0.25 millimeter.

In 4 of the 11 plants in which both bud growth and node potentials were measured, the buds did not start to grow out until 60 or more hours after decapitation. However, the node potentials in these plants showed the typical change after decapitation and were therefore diminished or inverted for 40 or more hours before any observable increase in the length of the buds. In 7 of these 11 plants the buds started to grow comparatively soon after decapitation. In 4 of these 7 plants both buds grew out; and in the remaining 3 plants only one of the buds definitely grew out. In every one of the 7 plants referred to above the node potentials definitely diminished before the buds started to grow. However, a given bud may start to grow before the corresponding node potential has become inverted. This is illustrated in B, figure 4; bud P_1' started to grow before the node potential A_1P_1' had become inverted, although this potential had diminished and was of comparatively small magnitude.

In 4 plants in which both buds grew out, the node potentials A_1P_1 and A_1P_1' followed one another fairly closely from the time of decapitation to the end of the experiment. This is illustrated in A, figure 4. On the other hand, in the 3 plants in which only one bud definitely grew out, there was a marked difference between the node potentials A_1P_1 and A_1P_1'. In each of these plants the node potential corresponding to the inhibited bud was definitely less positive than the node potential corresponding to the growing bud. In two of these plants the inhibited bud did not increase in length during the entire experiment. However, in the third plant, illustrated in figure 5, the "inhibited" bud started to grow, but became inhibited before the end of the experiment. In this plant the node potential A_1P_1, cor-

responding to bud P_1, returned to the normal orientation simultaneously with the inhibition of bud P_1. Later the node potential A_1P_1' also returned to the normal orientation. In fact, as can be seen from an examination of the results presented in table III, the node potentials frequently return to normal after the release of the buds.

TABLE III

COMPARISON OF THE NODE POTENTIALS AND GROWTH OF THE BUDS IN DECAPITATED PLANTS. COLUMNS 5 AND 6 REPRESENT THE LENGTH OF THE BUDS IN CENTIMETERS. COLUMNS 7 AND 8 REPRESENT THE INCREASE IN LENGTH OF THE BUDS DURING THE FOUR-HOUR INTERVAL. COLUMN 9 GIVES THE POTENTIAL DIFFERENCE FROM P_1 TO P_1'

PLANT	A_1B_1	A_1P_1	A_1P_1'	P_1	P_1'	$\triangle P_1$	$\triangle P_1'$	P_1P_1'
	mv.	*mv.*	*mv.*	*cm.*	*cm.*	*cm.*	*cm.*	*mv.*
1	− 9	− 9	+ 4	0.50	0.55	0.00	0.08	+13
2	+ 5	−10	+ 6	0.70	2.50	0.00	0.30	+16
3	−31	−30	−14	0.48	5.70	0.00	0.70	+16
4	−20	− 3	+14	0.75	9.50	0.00	1.10	+17
5	+13.5	− 9	+ 5	8.30	16.80	0.05	1.70	+14
6	+ 4	−11.5	−12.5	1.30	1.65	0.14	0.16	− 1
7	+21	− 5	− 3	4.00	6.20	0.20	0.40	+ 2
8	− 6	−15.5	−12.5	6.85	5.35	0.75	0.85	+ 3
9	+ 7	+ 5	+ 5	8.60	9.70	0.74	0.88	0
10	−20	− 3	− 5	7.30	12.30	0.55	0.76	− 2

For further confirmation of the relation between the inhibited bud and the corresponding node potential, 10 decapitated plants were selected, 5 of which had one bud growing and the other bud inhibited, while the remaining 5 had both buds growing. The node potentials and the increase in length of the buds of each plant were measured for 4 hours. This work was done in the greenhouse, and because of the variation of temperature during the day, the experiments were performed at night. The temperature did not usually vary more than one or two degrees during any experiment. The temperature limits for this group of experiments were from 20° C. to 24° C. In this series of experiments the length of the buds was measured with a horizontal microscope, giving an accuracy of about 0.1 millimeter. The results of these experiments are shown in table III. The average values of each of the node potentials are given in the second, third, and fourth columns. Columns 5 and 6 give the initial length of the buds in centimeters, and columns 7 and 8 give the increase in length of the buds during the four-hour period, also in centimeters. It can be seen from columns 7 and 8 of the table that, in the first 5 plants, the bud P_1 remained practically or completely inhibited throughout the experiment, while bud P_1' definitely increased in length. On the other hand, in the last 5 plants, both buds increased in length. In the first 5 plants, A_1P_1, corresponding to the inhibited bud, was

definitely more negative than A_1P_1', corresponding to the growing bud. In contrast with this, in the last 5 plants the average node potentials were of approximately the same magnitude. To clarify these results the potential difference between P_1 and P_1' was calculated from A_1P_1 and A_1P_1' and is given in the last column of the table. That this procedure is permissible has been amply demonstrated by LUND and BUSH (**13**), RAMSHORN (**16**), and CLARK (**4**). It may be pointed out here that the writer has tested this principle in *Phaseolus* and has found that it always holds. It can be seen that P_1P_1' is definitely positive in all of the first 5 plants, while in the last 5 plants it is comparatively small. That is, in plants with both buds growing out, the node potentials A_1P_1 and A_1P_1' were of approximately the same magnitude, and therefore P_1P_1' was of small magnitude. In plants with one bud inhibited and the other one growing, the node potential corresponding to the growing bud was definitely more positive than the node potential corresponding to the inhibited bud. For example, if bud P_1 was inhibited and bud P_1' was growing, A_1P_1' was more positive than A_1P_1 and therefore P_1P_1' was definitely positive. Since these last experiments showed a definite correspondence between the electrical polarity from petiole to petiole and the conditions of the buds, the question arose as to whether or not there was also a correspondence between the electrical polarity of the buds themselves and the state of the buds.

In order to answer this question, 8 plants were chosen, 4 of which possessed buds of approximately equal size, while in the other 4 one bud was definitely larger than the opposite bud. In these experiments contacts were placed on the buds 1.5 centimeters from the center of the corresponding node. Measurements were taken between A_1 and these contacts. In table IV, A_1C_1 refers to the potential difference between A_1 and the contact on bud P_1; similarly A_1C_1' refers to the potential difference between A_1 and the contact on bud P_1. Measurements were taken at night in the greenhouse every 15 minutes over a 4-hour period and each value in the table represents the average for this period. The temperature limits for this series of experiments were from 18.5° C. to 21° C.

It can be seen in this table that A_1 is definitely positive in every case to the contact on the bud. In each one of the first 4 plants in which a large difference in the size of the buds occurs, the potential difference represented by A_1C_1 (C_1 representing the smaller bud) was less positive than the other potential difference represented by A_1C_1'. This relationship is similar to the one found between the size of the buds and the node potentials A_1P_1 and A_1P_1'. The average potential difference between C_1 and C_1' was calculated from A_1C_1 and A_1C_1' and is given in the last column. This potential difference is definitely positive in the first 4 plants, *i.e.*, the base of the smaller and more inhibited bud is positive to the base of the larger bud. In the last 4

TABLE IV

COMPARISON OF THE POTENTIAL DIFFERENCES BETWEEN A_1C_1, A_1C_1' AND THE SIZE OF THE CORRESPONDING BUDS, P_1 AND P_1'. C_1 AND C_1' WERE CONTACTS PLACED ON THE BUDS 1.5 CM. FROM THE CENTER OF THE NODE. THE LAST COLUMN REPRESENTS THE POTENTIAL DIFFERENCE FROM THE BASE OF THE SMALLER BUD TO THE BASE OF THE LARGER BUD

PLANT	A_1C_1	A_1C_1'	P_1	P_1'	C_1C_1'
	mv.	*mv.*	*cm.*	*cm.*	*mv.*
1	+10	+34	3.1	12.2	+24
2	+13	+24	8.3	16.8	+11
3	+28	+37	8.6	15.0	+ 9
4	+19	+32	5.4	11.0	+13
5	+45	+46	5.6	8.5	+ 1
6	+42	+48	8.6	9.7	+ 6
7	+38	+36	4.3	4.5	− 2
8	+40	+32	4.7	5.2	− 8

plants, the actual magnitudes of the potential differences of A_1C_1 and A_1C_1' are greater than in the first 4 plants. On the other hand, the difference in magnitude between A_1C_1 and A_1C_1' is definitely less; that is, C_1C_1' is of smaller magnitude in the last 4 plants. This relationship again parallels that found between the node potentials A_1P_1 and A_1P_1' and the size of the buds.

The question then arose, is there a definite electrical polarity between the apical region of a large growing bud and a homologous region of the opposite smaller bud? Four plants were chosen that possessed buds of unequal size; in each case the large bud was over twice the size of the smaller bud. Contacts were placed on the stem directly below the apex of each and the potential differences were measured between these two loci at 15-minute intervals for a 4-hour period. These experiments were also performed in the greenhouse at night. The temperature limits were from 19.5° C. to 21° C. The apical region of the larger bud was found to be strongly negative to that of the smaller bud. The average potential differences for the four plants were −32, −37, −42, and −51 millivolts respectively. It is well known that the inhibitory influence travels from the growing bud to the inhibited bud. Since the inhibitory influence travels downward in intact plants, the question then arose, is there a similar polarity between the apex and the base of intact plants?

Eight intact plants were selected with 2 nodes developed and with an average height of about 1½ feet. In each plant one contact was placed on the stem directly below the apex and another contact on B_1, directly below the first node, and readings were taken every half-hour over a 4-hour period. The experiments were performed at night in the greenhouse; the temperature limits were from 21° C. to 25° C. The averages of these readings are pre-

sented in table V. In all of the plants except the second, in this table, the apical contact was definitely negative to the basal one.

TABLE V

POTENTIAL DIFFERENCE IN MILLIVOLTS BETWEEN THE APEX AND BASE OF EIGHT INTACT PLANTS

PLANT	1	2	3	4	5	6	7	8
	mv.	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*
P.D.	−51	+4	−70	−41	−48	−14	−32	−28

The question then arose: Is there a continuous potential gradient along the stem or are there polarities that are oppositely oriented to the main one? For an answer to this question the polarity of the second internode, *i.e.*, B_2A_1, was calculated from the data presented in table IV of the previous paper (**17**) and is presented in table VI of the present paper. It will be noted that the apical region of the second internode is positive to its base in all but one of the plants, although it was of small magnitude in plant number six.

TABLE VI

AVERAGE POLARITY OF THE SECOND INTERNODE, CALCULATED FROM DATA PRESENTED IN TABLE IV OF PREVIOUS PAPER (**17**)

PLANT	1	2	3	4	5	6	7
	mv.	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*
B_2A_1	+22.1	+19.5	−0.7	+21.9	+18.3	+1.7	+20.4

EFFECT OF APPLIED CURRENT ON THE INHIBITION OF THE BUDS

It is well known that the inhibitory influence can pass through a dead region in the plant (SNOW, **21**). This was also found to be true in the plants used in this investigation. Therefore, since the potential differences are dependent on the living cells (**17**), the electrical current is not necessary for the transport of the inhibition, at least over short lengths of the plant. Nevertheless, the possibility remains that the bioelectric potentials may influence the transport of this inhibition in the living tissue. This is in a sense similar to the passage of the hormone in the oat coleoptile. The hormone can pass from the decapitated tip through agar to the body of the coleoptile. The hormone apparently passes through the agar by diffusion but the transport of the hormone in the coleoptile itself is too rapid to be accounted for in terms of ordinary diffusion (VAN DER WEIJ, **26**).

Current from an external source was applied to intact plants in various ways, in order to find out whether or not an applied current could release the inhibition of the buds. The results were negative as far as the release of the buds is concerned, and are briefly summarized in the following paragraphs.

The strength of the currents varied from 1 to 50 microamperes and were sent through the plant with contacts similar to the ones used for measuring the potential differences. They were washed at regular intervals to prevent the products of electrolysis from coming in contact with the plant. The currents were sent in the following directions: A_1 to B_1, B_1 to A_1, A_1 to P_1, P_1 to A_1, P_1 to P_1', B_2 to A_1, and A_1 to B_2. The buds never grew out unless the stem between the apex and the node was killed and had dried out, and then only after the buds had grown out in control plants decapitated at the same time the current was first applied to the intact plant.[2] If only a short portion of the stem was killed, the buds remained inhibited. Sometimes the injury extended to the buds themselves and killed them (failure to regenerate after decapitation). Currents up to 5 microamperes produced little injury up to the time that the buds of the decapitated control plants started to grow out.

Although the applied current did not release the inhibition of the buds, several other effects of interest were observed. Injury always occurred first at the locus at which the current entered, even when these loci through which the current was sent were continually washed. This observation is similar to that of SCHECHTER (19) on the polarity of the lethal action of electric currents on *Conocephalus*. When current was sent from P_1 to P_1', epinasty occurred in the petiole toward the positive pole, and hyponasty in the one toward the negative pole. This effect was most pronounced in young intact plants, definite effects being produced within 6 hours by a current of 50 microamperes and within 20 hours by a current of 5 microamperes. It was also observed that when current was sent from petiole to petiole of the first node of young plants with a growing second internode, bending occurred in the second internode (a region through which presumably no current was passing). The internode would first bend with the convex side towards the positive contact, *i.e.*, the apex itself would move toward the petiole on which the negative contact was placed. The bend would later become reversed, the convex side moving toward the negative contact, and eventually (while the current was still flowing) the stem would become straight again. With a current of 50 microamperes, the stem would show a definite bend within 10 minutes after application of the current, and the whole process would last for about 2 hours. Currents of 5 microamperes would produce a definite bend within 30 minutes.

[2] Injury appears sooner at the loci where the current enters and leaves the plant than in the stem or petiole in between the contacts. Therefore, except in preliminary experiments, current was not actually sent at these loci but at a region 4 centimeters from the node, *i.e.*, 3 centimeters from the original contacts; and when the first signs of injury appeared the contacts were shifted slightly.

Discussion

The experiments presented in this paper indicate that there is a positive correlation between the electrical polarities and the inhibition and regeneration of the buds. However, many more experiments need to be performed before anything approaching a complete picture of the patterns of bioelectrical potentials in this plant can be presented. Nevertheless several definite relationships have been brought to light by these experiments. The fact that decapitating the plant produced an almost immediate effect on the node potentials which lasted for about an hour demonstrates that events happening at one locus of the plant can definitely modify the electrical processes at a distant locus. The hypothesis presented itself that this particular variation in the node potentials may in some way be a factor in the release of the inhibited buds. Aside from other considerations, it is evident from these experiments that this increase in the magnitude of the node potentials is not a necessary prerequisite for the regeneration of the buds of that particular node, since in many plants decapitated above the third node the buds of the first node regenerated as rapidly as those of the higher nodes, whereas the effect of decapitation of the node potentials was small at the second node below decapitation, and entirely absent in the third node below. Also, crushing the stem produced a response somewhat similar to, though of not as great magnitude, the response to decapitation; and the buds remained inhibited for long periods afterward. From these considerations one can at least tentatively conclude that this immediate response of the node potentials to decapitation is not directly related to bud regeneration.

The experiments on the later effects of decapitation indicate that there is a definite relationship between regeneration and the orientation and magnitude of the potential differences. One of the interesting observations on the later effects of decapitation on the node potentials is that they diminish almost to zero, or become inverted before visible elongation of the buds occurs.

The results on the orientation of potential differences in this plant tend to confirm WENT's hypothesis that the apices of this group of plants are negative to their bases.[3] He has also postulated that the negative radical of the hormone is transported electrophoretically towards the positive pole. The fact that the orientation of the potential difference in the second internode is opposite to the main polarity of this plant indicates on first inspection that the hormone could not be transported in the manner postulated by WENT in this internode. However, if one assumes that these potential differences existing in the plant give rise to currents and that a current flows downward, say in the region of the nodes, then there must obviously be a return current

[3] RAMSHORN (16) has reported what amounts to an apical positivity for several plants. In this connection it may be pointed out that both WILKS (27) and CLARK (4), working on the oat coleoptile, have reported an apical negativity in this plant.

flowing in the opposite direction. The experiments on the internal distribution of polarities suggest that the return circuit for current flowing upward in the region of the node is through the cells themselves. Obviously it becomes difficult to decide, from potential difference measurements alone, in what direction the hormone could be transported by these currents unless the exact path through which the hormone is transported and through which the current flows is known.

The observation that applied currents did not release the inhibition of the buds indicates that the bioelectric currents may not play a rôle in the longitudinal transport of this inhibition. In this connection it may be pointed out that CLARK (4) has presented evidence to show that the longitudinal transport of the hormone in the *Avena* coleoptile is probably independent of the measured bioelectric potentials. However, CLARK points out that there may be some subtle relationship between the bioelectric potentials and the transport of the hormone that escaped detection by his experimental methods.

The writer feels that certain considerations must be kept in mind when interpreting the results of applied current on living organisms. If we assume that the potential differences give rise to currents, then, as has been pointed out above, there must be a return flow of current. It is obvious that it would be impossible to duplicate exactly a particular pattern of currents in a complex tissue by the application of a current from an external source. An E.M.F. from an external source when applied to the plant will tend to send current in the same direction throughout that portion of the plant to which it is applied. Therefore it is possible to duplicate at best only one direction of current flow while at the same time the applied current is of necessity opposing the return flow of the inherent current. Thus while the duplication of one portion of the electrical pattern may accomplish some result that that portion of the inherent current may itself accomplish in the normal plant, this applied current is also producing an abnormal physiological state in adjoining regions. It is therefore interesting that even currents of small magnitudes do produce visible injury when applied to the plant.

The fact that certain morphological changes similar to those produced by the hormone are produced by the application of small currents, *i.e.*, epinasty, hyponasty, and bending, indicates that the bioelectric currents may in some cases alter the distribution or effect of the hormone in this plant, or in some other way affect the growth processes. The work of BRUNNER and AMLONG (3), KOCH (7), RAMSHORN (16), WILKS (27), and others indicates that there is some causative relationship between the bioelectric potentials and the growth process in the phenomena of phototropism and geotropism. However, not all of the results of these workers are consistent with the hypothesis that bioelectric potentials transport the hormone to the

measured positive pole. CLARK (4) has demonstrated that the hormone is transported in agar blocks only under comparatively large potential gradients.

Summary

1. The internal electrical polarities in the region of the nodes were found to possess the same orientation as the external ones.

2. Decapitation of a plant caused a large increase in the magnitude of the node potentials in the node directly below the point of decapitation, a small effect on the 2nd node below, and no effect on the third node below. Cutting a portion from the stem of a decapitated plant produced a similar response, but of smaller magnitude. Crushing the stem of an intact plant also produced a somewhat similar response to decapitation, although of smaller magnitude.

3. After decapitation, the node potentials returned to their former magnitude in about one hour and then steadily decreased until they were either inverted or of small magnitude. This decrease in the node potentials always preceded the first signs of regeneration of the buds.

4. In decapitated plants with both buds growing, the potential difference between the base of the petiole on the side of the growing bud was found to be negative to the base of the petiole corresponding to the inhibited bud; while in plants with both buds growing, the potential difference between the loci were found to be of small magnitude. Similarly, in decapitated plants the base of the growing bud was found to be negative to the base of the inhibited bud; and in plants with both buds growing, this potential difference was of relatively small magnitude. It was also found in decapitated plants that the top of the growing bud was negative to the top of the inhibited bud.

5. In intact plants, the apex was found to be negative to the base.

6. In intact plants, the apex of the second internode was found to be positive to the base of this internode.

7. Currents applied in various ways across the nodes of intact plants failed to release the inhibition of the buds except after a portion of the stem was killed and had dried out. Certain effects of the applied current not directly related to bud inhibition were observed.

8. In the discussion it is concluded that the response of the node potentials to the immediate effect of decapitation is probably not a causative factor in the regeneration of the buds. It is pointed out that there is a definite correlation between the inhibited and regenerating buds and the bioelectric potentials. The interpretation of the experiments on the effect of applied current is discussed.

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UPWARD MOVEMENT OF INORGANIC SOLUTES AS AFFECTED BY A GIRDLE[1]

HARRY F. CLEMENTS AND CHARLES J. ENGARD
(WITH NINE FIGURES)

Introduction

Much evidence has been obtained during the last few years relative to the question of the path of the upward movement of the greater portion of the inorganic solutes in plants. There appears to be little doubt that the xylem is the tissue chiefly concerned with this function. The purpose of this work, in addition to contributing more evidence to this conclusion, is to determine by use of a number of plants some of the effects which a girdle imposes upon the rise of inorganic solutes, and the relation of xylem anatomy to the ease with which solutes pass a girdle, a relationship first suggested by CLEMENTS (**2**).

Historical

The historical development of this subject has been outlined in a previous paper (**2**). Briefly, during the last fifteen years CURTIS (**4, 5, 6**) has developed the idea that the phloem tissue, and not the xylem, is chiefly concerned with the movement of inorganic ions upward.

CLEMENTS (**2**), in 1930, presented data definitely showing that there is a great upward movement of inorganic solutes past a girdle. Further, he indicated the error in CURTIS's experiments as well as in the latter's interpretative logic, pointing out that his failure to have proper controls rendered his experiments irrelevant to the conclusion that the phloem is the path of inorganic solute translocation, and that he unwittingly demonstrated that the girdle affects the rise of salts in part through its effects on the xylem. CLEMENTS showed further that while the quantity of salts which passed the girdle was very large there was a retarding effect caused by removing a strip of phloem and the consequent exposure of the current year's xylem. This retarding effect, at least in part, appears dependent in magnitude upon the distribution of tracheae in the seasonal development of xylem.

More recently, HOAGLAND and BROYER (**8**) demonstrated rather clearly that the xylem is concerned with the rise of the bromide ion. The rate of movement in the xylem appears to be maintained even after a girdle is made until some little while later, when it suffers a progressive retardation.

It is apparent from these investigations that since the salts travel with the transpiration stream in the xylem, they are subject to the same phe-

[1] Contribution no. 55 from the Botany Department of the State College of Washington.

nomena experienced by the transpiration stream. The results of the experiments set forth in this paper verify that relationship.

Methods

Eight campus trees and shrubs, and two fruit trees, were used in these experiments. The trees and shrubs were poplar (*Populus tremuloides*), willow (*Salix* sp.), dogwood (*Cornus stolonifera*) growing in two different localities, mountain ash (*Sorbus americana*), Russian pea (*Caragana arborescens*), lilac (*Syringa vulgaris*), honeysuckle (*Lonicera* sp.); and the fruit trees, apple (*Pyrus malus*, variety unknown) and crabapple (*P. malus*). These latter trees were growing under unirrigated conditions.

Two- or three-year-old stems were cut off about 1 cm. above a lateral bud and a ring of bark, including phloem and cambium, removed about 4 cm. below the bud or buds. The ring itself was about 0.5 cm. wide. The exposed xylem was protected as much as possible by grafter's wax. Controls taken at the time of girdling (beginning controls) consisted of similar stems removed at a point corresponding with the position of the girdle on the girdled stem. Another set of controls (end controls) was left on the plants to be removed when the girdled stems were collected.

The girdling was done and the beginning controls taken on April 18, 1936, just as the buds were beginning to develop. The beginning controls, six to fifteen for each plant, were taken to the laboratory and prepared for analysis. The girdled stems and their end controls were collected when they had passed their maximum rate of growth. The number of stems or "cuttings" was noted for each plant.

In all cases, the samples were dried in an oven at 80° C. and then ground to 40-mesh fineness in a power mill after weighing to determine total dry weight. Total ash content was determined upon duplicate samples blasted to constant weight in a muffle furnace. The ash contents are reported as percentages of the dry weight and as milligrams per cutting. Total nitrogen was determined by the usual Kjeldahl method, with no modification for nitrates and nitrites, and reported as percentages of the dry weight and as milligrams per cutting.

The anatomical work was done in the following manner: 1-cm. pieces of 2- or 3-year-old stems of each plant were cut and put immediately into turtox. Later, hand sections were made, stained in safranin, dehydrated in alcohol, and mounted in balsam by the usual method. Projection drawings were made from these sections.

Results

A discussion of work of this kind cannot be complete without some mention of the ecology of the region in which the plants were grown. The region has a bunch-grass climax. Only 14.68 inches of rain fell during the year in

which these studies were made, although the average approximates 21 inches per year, most of which falls in winter and spring; hence the normal growing season, especially June, July, and August, is dry. The atmosphere becomes extremely dry (as low as 5–10 per cent.) and the light intensity at this altitude often attains a magnitude of 12,000 foot candles; hence the transpiration rate is excessive. The trees on the campus have been introduced, and will maintain growth only when water is supplied. The trees used in this experiment, growing in a region which will not naturally support even the larger shrubs, are often handicapped by the inability of the root systems to provide water rapidly enough to prevent wilting. The occasional lack of water, a consequence either of erratic sprinkling or excessive demand, is manifested in the xylem of many of the trees by the formation of false rings (see *Populus*, (fig. 8), and *Pyrus* (fig. 3). Plants growing under such adverse conditions maintain a precarious balance with the environment, and any further inhibiting factor introduced into such a plant will be amplified under these conditions. Speaking teleologically, a tree in this region has an intense struggle for existence and reacts very strongly to girdling.

The girdled stems and controls of *Lonicera* were cut and brought into the laboratory on May 27, by which date they had made excellent growth. Both opposite lateral buds had developed on each stem, and the resulting growth attained a length of approximately 30 cm. The apple and crabapple stems were taken on June 28, when they apparently had made their maximum growth. Growth in both plants, as well as that of the girdled and ungirdled stems of each, was very good. On July 8, the remaining cuttings were collected. Of these, dogwood A[2] made the best growth, the girdled stems nearly equaling the ungirdled stems in appearance and size. The girdled stem of dogwood M made about half the growth of the ungirdled stems, and were visibly affected by the girdle. Poplar, willow, and lilac made only mediocre growth, and the girdled stems of Russian pea made very little growth. Only one girdled stem of mountain ash grew, and this but slightly. Of all the plants studied this one showed the severest reaction to the girdle. The analytical data are tabulated in tables I and II.[3]

Inspection of these data permits two fundamental conclusions: (1) the girdle does not *stop* the upward movement of inorganic salts, but, on the contrary permits the passing of large quantities of mineral salts in the xylem of the plant; and (2) treatment being the same in all plants, the response to the girdle seems to be characteristic of (a) the particular plant and (b) the particular site.

[2] Dogwood in two localities was used. These are designated "A" and "M" to indicate that from each locality.

[3] A "shoot" or "cutting" (tables I, II) is taken to be one girdled stem cut off at the girdle, or one control stem cut off at a point corresponding to the point of a girdle on the girdled stems.

TABLE I

TOTAL ASH CONTENT

GENUS	NUMBER OF SHOOTS	DRY WEIGHT PER SHOOT	TOTAL ASH AS PERCENTAGE OF DRY WEIGHT	ASH PER SHOOT
		mg.	%	*mg.*
Salix				
1st control	15	633	2.30	14.56*
Girdled	10	920	5.34	49.13
2nd control	6	5160	7.03	362.75
Lonicera				
1st control	10	980	5.03	49.29
Girdled	8	3375	6.91	233.21
2nd control	7	4957	6.00	297.42
Populus				
1st control	15	831	4.46	36.26
Girdled	24	1254	5.15	64.58
2nd control	10	1900	7.35	139.65
Syringa				
1st control	15	1026	4.80	49.25
Girdled	12	4216	2.66	112.15
2nd control	12	9435	3.29	310.41
Pyrus (apple)				
1st control	16	2312	4.66	107.74
Girdled	11	5636	5.19	292.50
2nd control	8	4312	6.08	262.20
Pyrus (crabapple)				
1st control	22	1341	4.71	63.16
Girdled	16	4163	5.01	208.51
2nd control	12	6916	5.36	370.69
Cornus M				
1st control	13	679	2.07	14.06
Girdled	12	2475	4.40	108.90
2nd control	6	5833	7.49	436.89
Cornus A				
1st control	13	1115	2.09	23.30
Girdled	12	5708	5.22	297.90
2nd control	5	9080	6.77	614.72
Caragana				
1st control	15	1466	2.52	36.94
Girdled	12	2141	3.64	77.93
2nd control	8	2787	5.40	150.50

* The amount of ash which passed the girdle in each case is the difference between the ash content of the girdled shoot and that of the first control, and, under equally ideal conditions for all species, seems to be correlated with the effect of the girdle on the xylem. The difference between the ash content of the girdled shoot and that of the end control is a measure of the effect of the girdle on the growth of the shoot, considering all factors influencing the rate of growth; but under ideal conditions would stand in an inverse relationship with the difference between the first control and the girdled shoot.

Considering now the first of these conclusions, it is readily evident from table I, that in all cases, the mineral materials continue to move into the shoots after the girdles were made. The largest increment of ash between the beginning control and the girdled stem is in dogwood A, which also exhibited the best foliar growth. The ash content of the girdled stems is about

TABLE II

TOTAL NITROGEN CONTENT

GENUS	NUMBER OF SHOOTS	DRY WEIGHT PER SHOOT	TOTAL N AS PERCENTAGE OF DRY WEIGHT	TOTAL N PER SHOOT
		mg.	%	*mg.*
Salix				
1st control	15	633	0.854	5.41*
Girdled	10	920	0.816	7.51
2nd control	6	5160	1.080	55.73
Lonicera				
1st control	10	980	2.240	21.95
Girdled	8	3375	2.176	73.44
2nd control	9	4957	1.856	92.00
Populus				
1st control	15	831	0.744	6.18
Girdled	24	1254	0.552	6.92
2nd control	10	1900	0.992	18.85
Syringa				
1st control	15	1026	1.576	16.17
Girdled	12	4216	0.928	39.12
2nd control	12	9435	1.632	153.98
Pyrus (apple)				
1st control	16	2312	0.888	20.53
Girdled	11	5636	0.904	50.95
2nd control	8	4312	1.194	51.49
Pyrus (crabapple)				
1st control	22	1341	0.880	11.80
Girdled	16	4163	1.248	51.95
2nd control	12	6916	1.509	104.36
Cornus M				
1st control	13	679	7.228	4.84
Girdled	12	2475	1.016	25.15
2nd control	6	5833	1.301	75.88
Cornus A				
1st control	13	1115	1.024	11.42
Girdled	12	5708	1.104	63.02
2nd control	5	9080	1.827	165.89
Caragana				
1st control	15	1466	2.376	34.83
Girdled	12	2141	1.920	41.11
2nd control	8	2787	2.517	70.15

* See footnote of table I.

13 times that of the beginning controls. Its nearest competitor on this basis, and in apparent growth, is dogwood M, which showed an ash content equal to approximately 8 times its check. *Lonicera* accumulated mineral materials in its girdled stems equaling 4.5 times that of the check; crabapple about 3.5 times the check; and the girdled apple stems a little less than 3 times their checks. The girdled stems of willow, and lilac approximately trebled their ash contents. The Russian pea and the poplar, the girdled stems of which had twice the ash content of the checks, had the smallest increment of ash of all plants used. *Sorbus* failed to grow. Thus there is a transport of inor-

ganic salts past the girdle, and obviously in the xylem, in quantities ranging from 13 down to 2 times the salt content of the beginning controls. It should be remembered that this salt movement was taking place in plants growing under difficult ecological circumstances.

Scrutiny of table II, which presents data on the total nitrogen content, reveals an increment gradient of the same pattern as that for the ash content. Here, as with the ash content, the two dogwoods yield the greatest increase in total nitrogen in mg. per cutting, the girdled stems of dogwood A showing a total nitrogen content of 5.8 times that of the beginning controls, and of dogwood M, 5 times the nitrogen content of the beginning controls. The girdled shoots of crabapple, *Lonicera*, and apple are next, with total nitrogen contents of 4.3, 3.5, and 2.5 times the content of the respective controls. Poplar and Russian pea are at the bottom of the list. The assumption here is, of course, that all nitrogen compounds present above the girdle were derived from nitrogen salts rising through the xylem, and synthesized in the leaf region above, a phenomenon known to be common among perennials (17). In *Populus* and *Caragana* the increase above the girdle is so small that it indicates a situation such as THOMAS (17) found, namely, that the inorganic nitrogen loses its identity in the roots and is changed to the organic, and as such may move through tissues other than xylem. This may, indeed, account for some of the poor growth made by some girdled shoots. It may be pointed out, however, that even the ungirdled end controls do not increase in nitrogen as much as they do in ash. Thus the total nitrogen per girdled shoot ranges from practically nothing in *Populus* to 5.8 times the nitrogen content of the beginning controls in *Cornus*.

These increases in the ash and total nitrogen content of the girdled stems are definite demonstrations of the thesis that large quantities of inorganic solutes rise in stems after the continuity of the phloem has been broken, and therefore lead to the conclusion that the xylem is the chief path of transport. That this conclusion is correct has been further verified by HOAGLAND and BROYER[4] who showed that xylem tissue carries the bromide ion, and, what is perhaps more significant, that the removal of a ring of bark did not greatly, if at all, affect the upward movement of this ion in the cotton plant for several hours, or in young citrus trees for much longer periods. This means, then, that a girdle, *per se*, in so far as it breaks the continuity of the phloem, does not affect the upward movement of salts, but that other factors, induced as a consequence of the girdling process, somehow interfere with the upward movement of the dissolved salts in the xylem. The dissolved salts are identified as being in the "transpiration stream," and would seem to be subject to the same forces.

[4] Private communication. The writers are also informed that some earlier work by Dr. F. C. STEWARD on movement of the bromide ion has been done, but the results have not yet been reported.

Turning now to the second conclusion derived from these studies, namely, that the effect of the girdle on the upward movement of salts appears to be characteristic of the species, one can arrive *a priori* at the conclusion that the distribution and size of tracheae and tracheids, and the cell wall thickness of water conducting tissues are important factors governing the ease, and therefore the rapidity with which the rising column of water with its dissolved salts can negotiate the girdle. It was this thesis which determined the course of experimentation pursued in this work.

A tracing of a projected cross-section of the xylem was made of each plant used in the investigation. These are shown in figures 1 to 9. They are correlated in table III with the data of tables I and II, and are found to

TABLE III

CORRELATION OF XYLEM ANATOMY WITH THE INFLUENCE OF A GIRDLE ON THE RISE OF ASH MATERIALS

XYLEM TYPE	GENUS	ASH PER SHOOT		
		1ST CONTROL	GIRDLED	INCREASE
		mg.	*mg.*	%
Diffused porous				
Shrub	*Cornus* A	23.30	279.90	1178.8
Shrub	*Cornus* M	14.06	108.90	674.5
Shrub	*Lonicera*	49.29	233.21	373.1
Tree	*Salix*	14.56	49.13	237.4
Tree	*Pyrus* (crab-apple)	63.16	208.57	230.2
Tree	*Pyrus*	107.74	292.50	171.5
Shrub	*Syringa*	49.25	112.15	127.9
Ring porous				
Shrub	*Caragana*	36.94	77.93	110.97
Tree	*Populus*	36.26	64.58	77.9
Tree	*Sorbus*	.....	.....	.....

correspond rather closely with the increment gradient of the ash and total nitrogen content. On the basis of the following observations, such a correlation might well be expected:

(1) The new ring of xylem is the chief functional region as regards sap rise, since it leads directly to the new regions of growth and activity, although some sap may rise in the xylem of the previous year, depending upon the quantity of gas held within the central cylinder (**10**).

(2) Because of an insufficient supply of elaborated foods from above, the production of new xylem below the girdle is considerably curtailed, while above the girdle the new xylem production is unimpaired because of an ample supply of carbohydrates moving downward from the leaves; in

fact, this growth above the girdle may even be stimulated under ideal conditions.

(3) The rising transpiration stream in the old xylem makes connection with the new xylem above the girdle by a lateral movement from the older vessels to the vessels of the new wood, especially where the distal portion of the experimental branch has been removed. Further, the girdling process, no matter how carefully done, causes injury to the xylem. Even though the exposed xylem is protected by paraffin or grafting wax, there is some incapacitation of it that is caused not by mechanical injury but by a plugging of the xylem elements with air drawn in by suction tension (**10**), or by a general disorganization caused by the isolation of the living cells of the xylem, which die as a result of girdling, as is evidenced by a discoloration of the exposed tissue. Also, the reduction in the cross-sectional area of the water channel itself, or at least the prevention of its development at the place of the girdle, will be effective in reducing water movement. It must be remembered that, if a girdle is made in the spring, the xylem which is exposed is that which was sufficient to provide water for the previous season's growth, but not for the greater foliage development of the present year. This fact must be taken into account whenever girdled and ungirdled stems are compared, because the actual conducting volumes in the two cases are unequal. To obtain a figure for the relative conducting volumes, girdled and ungirdled stems of *Cornus* were taken. The girdles were made July 15, at which time the plants were approaching their greatest size. Six weeks later, these shoots together with the ungirdled controls were removed. The stems were cut (a fresh cut made under water to prevent air clogging) and water was sucked through 10-cm. lengths to a suction flask. One of the 10-cm. lengths included the girdle, while the control was taken in juxtaposition to the girdled portion. An average of several 3-hour tests showed that the ungirdled stems had conducted 33.8 per cent. more water than the girdled stem. Since, unlike the field conditions, the force was distributed equally over the whole cross-sectional area, there was no lateral movement in the ungirdled stem; so the result is no more than the measure of the relative cross-sectional areas of the conducting tissue. In diffused porous woods in which the vessels lie next to the new xylem, there may be a lateral movement of the stream farther into the old wood because of the plugging of the columns next to the girdle. In a case of this kind there would be two lateral movements, one inward below the girdle and the other outward above the girdle.

(4) The rapidity and ease with which a lateral transfer from xylem of a previous year to that of more recent development is effected is apparently dependent upon (1) the proximity of the tracheae of the old xylem to those of the new xylem; (2) the size of the tracheae; (3) the number of tracheae and other elements through which the laterally moving solutions must pass; and (4) the thickness of the cell walls of all xylem cells.

A consideration of the mechanism of the lateral movement must be included before going farther. While the xylem rays may be instrumental in moving foods and salts from xylem to phloem and *vice versa,* it seems improbable that a mass movement of water would take place through them sufficient to supply the amount used by the transpiring leaves; while such a movement is possible, at best it would be extremely slow. The possibility that cell wall pits are operative in such a lateral movement, a postulate of STRASBURGER (**16**), has been discredited by MACDOUGAL, OVERTON, and SMITH (**10**). The fact that in the majority of plants the pits are found sometimes exclusively on the radial rather than the tangential walls, while lateral movement still takes place, would seem to further verify this criticism. It should be emphasized that the lateral movement of water, in this paper, refers to the movement of water from older to newer xylem, not merely a movement tangentially about the stem.

There remain, then, two possible means of lateral movement; a lateral movement through leaf or branch traces (**10**), and a lateral movement through the cellulose gels which constitute the cell walls. MACDOUGAL, OVERTON, and SMITH (**10**) showed that no dye was moved laterally to outer xylem layers from inner xylem layers to which dye had been added through a bore-hole. They concluded that there is no lateral movement, and could not demonstrate that movement took place through the traces. As the method by which the stream is transferred from the older layers of xylem into the newer layers, they suggested that the central column of xylem has a conical terminal which "is surrounded by a thimble-shaped mass of wood whose upper half (longitudinal growth of the present season) is solid, and whose lower half (the portion formed laterally to the previous year's wood) is hollow. It appears, therefore, that in the two-year-old twig, water entering the first year's wood moves more or less vertically through it to the region where the second year's wood caps that of the first year." Thus, the "lateral" movement as postulated by MACDOUGAL, OVERTON, and SMITH (**10**) is actually a vertical movement caused by relayed suction forces of the transpiring leaves of the current year. The results of their dye experiments seem to be consonant with such a mechanism, but in this work the stems are cut off above the lateral bud, making all such vertical transfers impossible. How, then, can the developing bud receive the influx of salts except by a lateral movement of the rising transpiration stream directly across the xylem, either through the cell walls or the lamellae? There appears to be no alternative.

In order to test this hypothesis some shoots of *Cornus* and *Caragana* were girdled on July 14. The tops of these shoots were not removed as in previous experiments. Six weeks later these shoots were removed at a point several inches below the girdle. These shoots were plunged into water, and a second

cut was made to prevent air plugging. They were placed in a solution of dye. After four days, they were removed and the course of the dye movement was determined. The dye had moved past the girdle in *Cornus*, but, while at the girdle only those vessels at a distance from the outside were colored, immediately above the girdle the dye was found in xylem cells next to the cambium. *Caragana*, however, showed no passage of the dye above the girdle, even though it apparently was receiving water. Thus, a lateral movement in *Cornus* is demonstrated, which in a sense contradicts the conclusion of MacDougal, Overton, and Smith. This further indicates that a girdle incapacitates the exposed xylem even though the latter is not structurally destroyed, since only those vessels at a distance from the exposed xylem cells conducted the dye.

In an earlier publication, MacDougal (9) reports an experiment in which several small trees were girdled about September 1. A few days later a tree was cut, topped, and attached to a suction pump, with the base of the tree in fuchsin. The pump was operated at a vacuum of 74 cm. of mercury for 4 hours. At the end of the time 250 cc. of dye had been drawn through to a suction flask past the girdle. The dye had passed through in all layers of the xylem, with no variation of the conducting stream. Another of the girdled trees was cut a few weeks later and treated in the same manner. The time of pumping was from 3 P.M. one afternoon to 9 A.M. the next morning, and at the end of that time 200 cc. of dye had been drawn through at the normal rate of 8 cm. per hour. All layers of xylem were colored up to half the distance, and above that it was found only in the second and third layers of xylem, and "when the stem was bisected it was seen that the exposed wood formed during the current year had not conducted the dye, which had come up in this layer to the margin of the exposed zone, been blocked out of it across the girdle, and then *diffused into*[5] and followed it up many centimeters above, as has been noted."

In the system used in our experiments there is no equal tension on each xylem year because the stem is cut off. If the current development of xylem is inhibited below the girdle, the transpiration stream rising primarily in the previous year's xylem is drawn past the girdle laterally into the current xylem above the girdle by a "one sided" suction tension from the leaves. Obviously this situation is very different from that of the dye injection experiments of MacDougal, Overton, and Smith, and of MacDougal's experiment.

This lateral movement, seemingly a "soaking process," operates upon the cell wall which is regarded as a cellulose gel. From recent x-ray work by Sponsler (**12, 13**), Sponsler and Dore (**14**), Astbury, Marwick, and Bernal (**1**), and others much light has been thrown upon the structure of

[5] Italics ours.

the cell wall. Cellulose is regarded by these workers as a double anhydride of glucose of the empirical formula $C_6H_{10}O_5$, four of which are bound together to form the unit. The dimensions of the unit are 8.35 Å, 10.34 Å and 7.0 Å. The micelle of cellulose consists of elongated groups of 40 to 60 long chains, each being 50 to 80 hexan units in length, the anhydrides being held together by strong primary valence forces. Wood, however, is more than just cellulose. The primary or fundamental cell-membrane or fiber is cellulose, which is converted into wood by lignification or an "incrusting" of the cellulose with "lignin," a collective term referring to a group of heterogeneous organic substances.

These substances of carbohydrate derivation exist in wet conditions as gels, whose swollen volume is determined by the amount of water contained. The micelles of cellulose evidently are much closer when a fiber is dried than when it is swollen, according to FREY (7). CRAFTS (3) has presented a diagram showing cell walls much swollen in the natural condition and shrunken when partly dried or fixed for histological purposes. Further, cellulose swells more in solutions of electrolytes than in pure water, the reason being that "one ion is absorbed more than another. This separation of electric charges results in the stronger binding of the electric dipole of water; it also charges all of the cellulose micelles in the same sense, thus causing them to repel one another. This repulsion separates the micellar structure and allows water to enter, as it were, passively into the increased interstices" (11). The transpiration stream is, among other things, a weak solution of electrolytes. The fact that swelling takes place indicates that water is taken into the structure; and when a considerable "suction" force is applied at one side of the gel, it seems reasonable that water can be drawn through the avenues which are present as micellar interstices.

The thicker the cell wall, the more difficult will be the passage of solution through it, since a greater total-resistance will be encountered. If the lateral movement takes place across a great many small, thick-walled tracheids, the movement would be much slower than across a few thin-walled tracheids. But even where this lateral movement is comparatively easy, it is more difficult than a vertical movement through the lumina of the vessels.

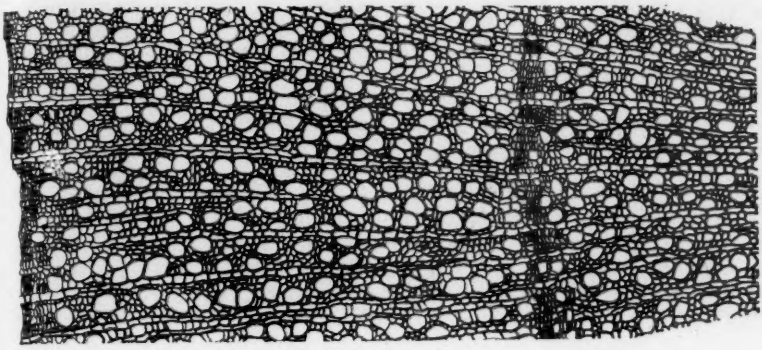
There are two general types of xylem as regards tracheal distribution: the "ring-porous" type, in which the tracheae are concentrated in the spring growth of xylem, forming a more-or-less definite ring; and the "diffuse-porous" type, in which the tracheae are scattered evenly throughout the whole annual ring. There are, of course, all degrees of intergradation between ring-porosity and diffuse-porosity, a "semi-diffuse" porous xylem representing a median where large and closely packed vessels are concentrated in the spring wood, with vessels becoming increasingly smaller and less frequent throughout the summer wood, with few or none in the late summer wood at the end of the year's growth.

The xylem of the plants used in this experiment represent a rather complete series of the above types, and, further, in one of them, *Sorbus*, the production of tracheae has been reduced to only scattered groups of six or eight cells per group, those generally in the spring and early summer wood. For convenience in correlating the xylem anatomy of these plants with their corresponding ash data the following grouping of the plants is used and the correlation is made with the analytical data in table III.

In this table, two groups are made: the plants with diffuse-porous xylem, represented by dogwood, apple, honeysuckle and willow; those with ring-porous xylem, represented by poplar, lilac, and Russian pea; and the mountain ash with its paucity of vessels but so arranged that it does not fit into either of the first two groups. On the basis of the ability of the xylem to carry the solutes when girdled, these plants arrange themselves in order of decreasing amounts as follows: dogwood A, dogwood M, honeysuckle, willow, crabapple, apple, lilac, Russian pea, poplar, and mountain ash. Figures 1 to 9 reveal that, on the basis of xylem structure with reference to tracheal number and distribution, the order from greatly diffused porous xylem to ring porosity is as follows: crabapple, dogwood A, dogwood M, apple, honeysuckle, willow, lilac, Russian pea, poplar, and mountain ash. Were it not for the fact that the two apple trees are out of line, the series would be exactly parallel. The behavior of the apple trees, however, is explainable when it is remembered that they were not watered, but were growing under difficult ecological conditions. This emphasizes the fact that whatever influences the transpiration stream also influences the movement of soil solutes, although not necessarily in proportion.

There is no sharp line of demarkation between diffuse-porous and semi-diffuse-porous, but for the sake of the grouping, crabapple, apple, dogwood, and *Lonicera* have been placed in the diffuse-porous group. If one inspects figures 1 to 4[6] one will have no doubt that crabapple and *Cornus* are diffuse-porous. In these two plants the vessels are numerous, large, and evenly dispersed. There are 6 or less tracheids separating the vessels of one annual ring from another in both of these plants. In crabapple, however, the vessels are much more numerous, and the tracheids separating them are fewer than in *Cornus*; for this reason crabapple has been placed first in the order, and *Cornus* second. In the apple, the vessels are less numerous than in crabapple, and much smaller than in crabapple and *Cornus*. Here, also, there is just a suggestion of departure from the strictly diffused porous type, manifested by a slightly greater concentration of tracheae in the spring wood. The vessels of the spring wood are also slightly larger than those of later summer. The tracheae of *Lonicera* (fig. 4) are scattered throughout

[6] The writers are indebted to DOROTHY ENGARD for her assistance in making the xylem drawings.



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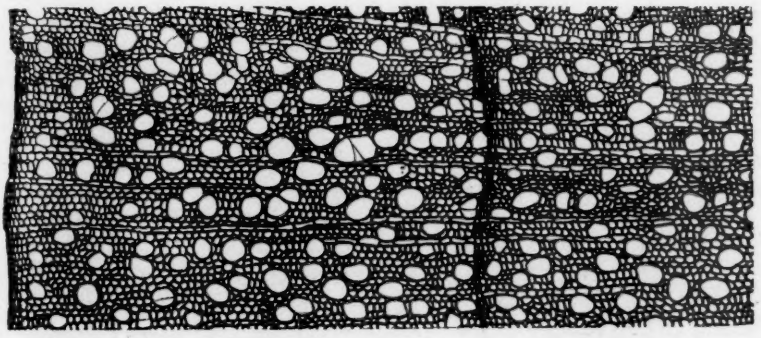

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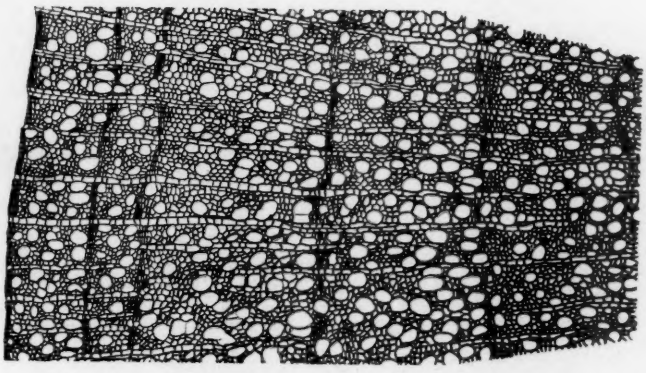


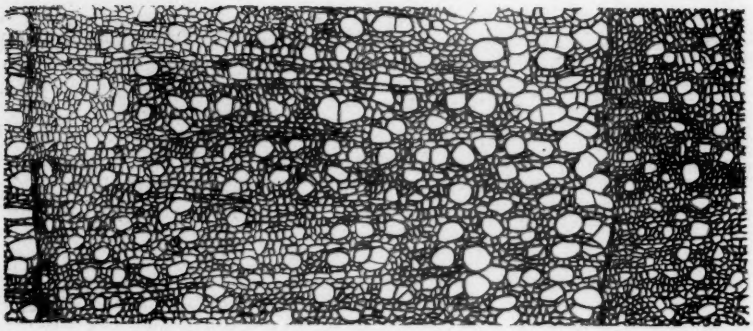
3

Projection drawings of cross-sections of secondary xylem

FIG. 1. Crab-apple (*Pyrus malus*).

FIG. 2. Dogwood (*Cornus stolonifera*).

FIG. 3. Unknown variety of apple (*Pyrus malus*).



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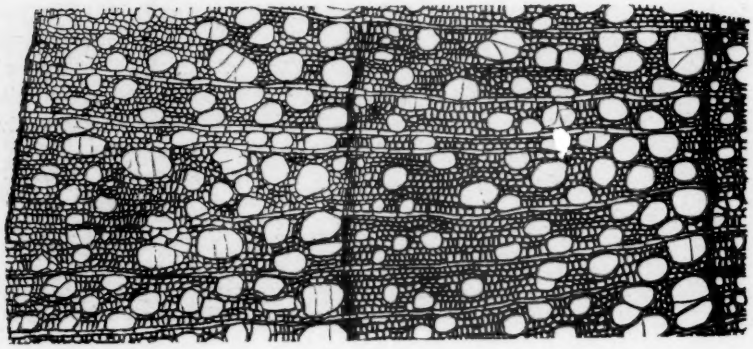

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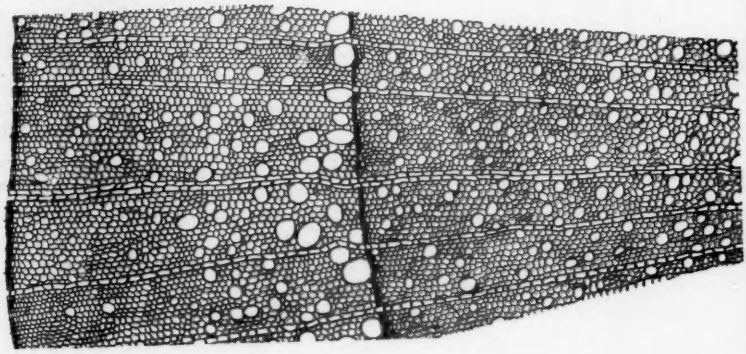


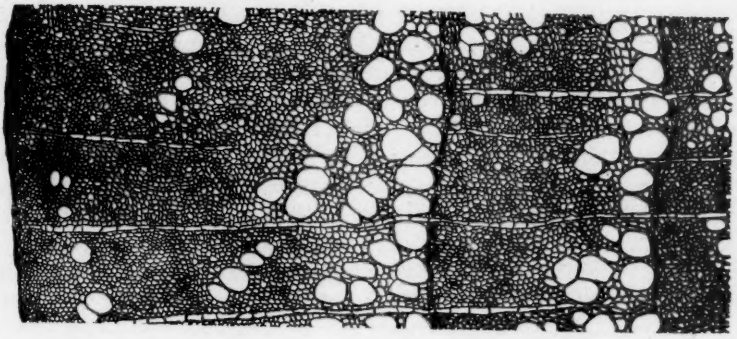
6

Projection drawings of secondary xylem

FIG. 4. Honeysuckle (*Lonicera* sp.).

FIG. 5. Willow (*Salix* sp.).

FIG. 6. Lilac (*Syringa vulgaris*).



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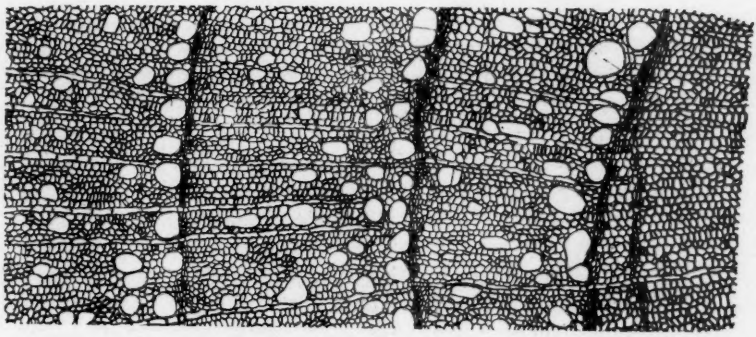

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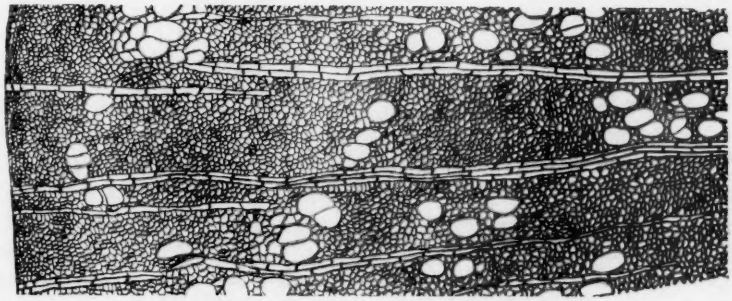

9

Projection drawings of secondary xylem

FIG. 7. Russian pea (*Caragana arborescens*).

FIG. 8. Poplar (*Populus tremuloides*).

FIG. 9. Mountain ash (*Sorbus americana*).

the ring, but there is a definite suggestion of ring porosity. The vessels of the spring wood are larger than those of the summer wood.

Salix and *Syringa* are representatives of the transition group—the semi-diffuse-porous group. The vessels of *Salix* (fig. 5) are more numerous and more evenly distributed than those of lilac, and very much larger. The spring wood of willow contains a great number of extremely large vessels which give it a marked degree of ring porosity. Lilac (fig. 6) is considered semi-diffuse-porous because of its scattered, small vessels in the summer wood. The late summer vessels are very small, and only a few are closer than 8 or 10 tracheids to the vessels of the new year ring. The tracheids are very thick-walled.

Caragana (fig. 7) and *Populus* (fig. 8) are definitely ring-porous, the majority of vessels being concentrated in the spring wood, with only a few in the summer wood. The total tracheal area of *Caragana,* however, far surpasses that of *Populus,* and there are some groups of vessels which actually border on the new annual ring; for these reasons *Caragana* has been placed ahead of *Populus* in the chosen order. Also, *Caragana* is a shrub which produces a large, fast-growing annual ring, whereas *Populus* is a tree which produces smaller annual rings.

Mountain ash (*Sorbus,* fig. 9), for which there are no analytical data, is placed last in this grouping. There is in the xylem of this plant no special region of tracheal concentration; but, instead, the tracheae appear in a limited number in small groups, which are located in the spring and summer wood. The tree is a rapidly growing one, producing a large annual ring (note fig. 9) which, however, is made up almost entirely of small, very thick-walled tracheids. The resistance offered to a laterally moving stream would be extremely great, and this fact, coupled with the adverse conditions under which these plants grow, permits of little or no accumulation of salts above the girdle; in fact, in all but one of the girdled stems, death by desiccation resulted in the portion above the girdle.

A further consideration of the data of tables I and II will reveal consequences of an excessive transpiration rate. The effects of high transpiration and lack of water working together can be noticed in the data by the general tendency of the end controls to have higher ash and total nitrogen percentages than the girdled stems. This is especially true of those plants which remained growing until July 8, which indicates that, as the summer and increasing drying continued, the girdle as it affects the xylem became the limiting factor in transport of solutes up the stem. These data, then, show two important facts: (1) that the total amount of solute passing the girdle has the xylem anatomy as the limiting factor; and (2) that the great transpiration rate working in conjunction with an insufficient water supply, forces the girdle to become even more the limiting factor in the transport

of water with its solutes. These facts can be demonstrated in the first case by comparing the beginning controls with the girdle, and in the second case, by comparing the girdled stems with the end controls. It is in this second case that drought and transpiration become the forces at play on the system.

As though paradoxical to the previous data, the end controls of the diffused porous plants with thin-walled tracheids and vessels (with the exception of *Lonicera*) showed the greatest increase of ash and total nitrogen over the girdled stems when compared with the semi-diffused and ring-porous plants. The series is reversed, with the end control of *Populus* showing the least increase in ash and total nitrogen over the girdled stem. However, just the proximity of the thin-walled vessels to the exposed area increases the likelihood of their being dried out, plugged, or disrupted by the intake of gas into the region about the girdle, thus incapacitating a considerable percentage of the area of the ascending water columns. In such ring-porous woods as *Populus* and *Caragana* by far the greater portion of the conducting area lies deep in the annual ring; and any plugging of the tracheids in the girdle region will result in very little decrease in the extent of translocational facilities of the plant. The thickness of the exposed cell walls will undoubtedly be a factor determining the extent of drying or of the intake of air into the xylem elements. Thus one would expect a greater percentage of reduction of upward movement in those plants whose xylem would *normally* facilitate upward movement of solutes, and a lesser percentage of reduction in the movement of solutes in the ring-porous woods.

There is, as was pointed out above, a direct correlation between the effect of the girdle and the conditions under which the plant is growing, a fact which can be illustrated by a comparison of *Lonicera* and *Cornus*. These possess very diffuse-porous wood with vessels in large numbers bordering on the cambium or on the spring wood of annual rings, and yet the results were quite different. Their respective ash contents are as follows:

	Girdled	End control	Difference
Cornus	108.9 mg.	436.9 mg.	328.0 mg.
Lonicera	233.2 mg.	297.4 mg.	64.3 mg.

The difference may be accounted for by the difference in conditions of growth of the plants. It will be remembered that the stems of *Lonicera* were harvested on May 27, less than 6 weeks after girdling, whereas the stems of *Cornus* were left on the plant until July 8. It has been stated that most of the rainfall of the region comes in the winter and spring. The month of May is usually the last to receive much rain, and the air is humid, the soil saturated as a result of winter snows and early rains. Conditions for growth in April and May undoubtedly parallel those of a normal mesophytic tree-bearing region, so it is to be expected that results obtained in this period for *Lonicera* are more likely to approach the results which would be obtained

under normal tree-growing conditions. The effect of the girdle would, under the latter conditions, be much less pronounced than that observed for the July collection presented here. It was noticed in July that the leaves of the *Lonicera* from which the material had been collected in May were drying out and curling, a consequence of desiccation. The differences between the end controls and the girdled shoots, therefore, appear to be larger than would be the case under more humid conditions.

The data indicate that dogwood A and dogwood M, although of the same species, reacted differently to the girdle. Their places of growth are quite different, however, dogwood A being on the campus, and exposed to sunlight intensity characteristic of the region; while dogwood "M" grows against the east wall of a campus building, and is exposed part of the day to the sun, the intensity of which becomes much greater than is normal by reflection from the wall. The intense heat increases the transpiration rate to a much greater degree than in the case of dogwood A, and because of this increased transpiration the deleterious effect of the girdle is greater.

It seems, therefore, that the differences between the end control and the girdled shoot may be considered to measure the effect of the girdle on the specific limiting factor which retards the growth of the particular plant. In some cases (*Cornus*, etc.) this seems to be high temperature; in some, water, (*Pyrus*); in some lack of nitrogen in the shoots (*Populus*, etc.). It is reasonable to suppose that, were all conditions ideal for each individual species, the difference between the girdled shoots and the end control would be considerably reduced (*Lonicera*); but only in the unusual case (*Pyrus*) would the girdled shoot exceed the ungirdled one in growth.

Summary

1. Data for girdling experiments are given for poplar (*Populus tremuloides*), willow (*Salix* sp.), dogwood (*Cornus stolonifera*) growing in two different localities, mountain ash (*Sorbus americana*), Russian pea (*Caragana arborescens*), lilac (*Syringa vulgaris*), honeysuckle (*Lonicera* sp.); and the fruit trees, apple (*Pyrus malus*, variety unknown) and crabapple (*P. malus*).

2. Shoots from each of these plants were selected in groups of three, one of these girdled, one removed at the time of girdling, and the third removed at the time the girdled shoot was removed. The difference in ash and nitrogen contents between the first and second shoots indicates the amount of each of these materials which passed the girdle; and the difference between the first and third shoots indicates the effect which the girdle, through its influence on the sum total of growth factors, imposes on the upward movement of the transpiration stream and its salts.

3. The data so obtained show, first, that a considerable amount of both

ash and nitrogen passed the girdle, thus further establishing the generalization that the upward movement of inorganic solutes takes place chiefly in the xylem; second, that the influence of the girdle is somewhat characteristic of the species and of the environment in which the plant is growing.

4. The effects of the girdle on the development of the parts above it seem to be as follows: (1) The removal of a ring of bark prevents the development of new xylem at that point, which, as growth above continues, becomes a constriction in the cross-sectional area of the conducting channel. (2) No matter how carefully a ring of bark may be removed even though no mechanical injury has been inflicted, and even though the wound is carefully covered, the xylem is affected in two ways: first, its living cells die as a result of their isolation from the living cells of the phloem; and second, as a consequence of this and of the actual exposure of the xylem, the outer layers of conducting cells lose their capacity to conduct water, and thus further limit the capacity of the xylem to conduct water with its dissolved salts. (3) The imposed girdle, as a result of the effects mentioned, makes necessary a transfer of water from one annual ring to another. The number, size, and distribution of the tracheae in the annual ring of wood seems to determine the rate with which the water with its dissolved salts can thus be transferred. A good correlation was found between tracheal distribution within the xylem of the plants studied and the amounts of minerals which passed the girdles.

5. It appears therefore that the girdle, as a break in the continuity of the phloem, does not affect the upward movement of salts; but rather that the girdle, as it affects the movement of water, influences the upward movement of salts in the xylem.

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SOME ABSORPTION SPECTRA OF LEAF EXTRACTS[1]

G. MACKINNEY
(WITH FOUR FIGURES)

Introduction

This study was originated in an attempt to account for the visible difference in appearance of toluenized leaves dried in air and in hydrogen. It was extended to include several other methods of leaf-killing, to determine the significance of changes noted in the absorption spectra of the leaf extracts. Marked differences, dependent upon the method of preparation of extracts, have been noted by STRAIN (5) for certain of the xanthophyll components, and it was thought that equally striking changes in the chlorophyll components might be found. Because many of these differences can be ascertained only by the use of an adsorbent, adsorption studies have been made to supplement the spectroscopic data.

Few methods of adsorption—none, so far, to the writer's knowledge, for plant pigments—are adequate for detecting quantitatively the differences in the ratios of the various components for a leaf under study. In spite of this drawback, the adsorbent furnishes a useful check on changes which may take place in the pigment complex, and in many instances prevents differences in the spectroscopic data from being ascribed to wholly erroneous causes.

Inulin has been found in this laboratory to be an extremely useful adsorbent for separating the *a* and *b* components of chlorophyll from crude leaf extracts in petroleum ether. It has two apparent advantages over sucrose, its markedly lower hygroscopicity and the fact that purification of the leaf extracts is unnecessary.

Analysis of absorption spectra data

Part I of this paper deals with an analysis of the absorption spectra curves of eighty-four extracts from ten different sources. The curves were examined as to constancy of shape, and where significant discrepancies appeared, explanations were sought, either on the basis of fluctuations in the ratios of chlorophyll *a* to chlorophyll *b*, or on the assumption that degradation products were present. Part II contains supplementary evidence by the TSWETT adsorption technique with inulin as an adsorbent, for seven of these sources.

PART I

Detailed studies were made of the absorption spectra of crude leaf extracts, in 80 per cent. aqueous acetone, for the region 6800 to 6000 Å, where

[1] Conducted under the auspices and in the laboratory of the Division of Plant Biology, Carnegie Institution of Washington.

carotenoid absorption does not obscure the curves for the chlorophyll components. The transmissions were measured on the spectrophotoelectric apparatus constructed by SMITH (**4**), and the double logarithm of the reciprocal of the transmission was plotted as a function of the wave length. In this way, significant differences were readily noted by comparing the shapes of the curves.

The following leaves were studied: clover (*Trifolium repens* L.), sunflower (*Helianthus annuus* L.), begonia (*Begonia semperflorens* Link & Otto), sorrel (*Rumex acetosella* L.), barley (*Hordeum vulgare* L.), ivy (*Hedera helix* L.), tobacco (*Nicotiana tabacum* L.), eucalyptus (*Eucalyptus globulus* Labill.), Virginia creeper (*Parthenocissus quinquefolia* Planch.). Through the courtesy of Professor C. B. LIPMAN, the writer is enabled to include observations on a blue-green alga of peculiar interest.

The sap of the photosynthetic tissue of sunflower is sometimes alkaline, that of barley mildly acid, while begonia, sorrel, and Virginia creeper are rather strongly so. Tobacco has a high starch content, and drying is difficult. In this study, leaves with visible anthocyanin were excluded, as these pigments would introduce an additional variable in an already complex situation. Otherwise, it may be suggested that the material selected represents a fairly wide range of leaf types.

Five general methods of leaf-killing were employed:

1. Drying: a. at room temperature *in vacuo*.
   b. at 45 to 50° C. in air.
2. Direct solvent extraction (with acetone).
3. Freezing at – 80° C.
4. Dipping in boiling water.
5. Anaesthetics: a. toluene in air.
   b. toluene in hydrogen.
   c. chloroform in air.

In all cases, the leaf material (2- to 5-gm. samples, fresh weight) was triturated in a glass mortar, sometimes with the aid of ground glass (40 to 80 mesh), which apparently had no deleterious effect, and was then completely extracted with 80 per cent. acetone. The extracts were filtered and made to volume (50 or 100 cc.) from which 2- or 4-cc. aliquots were drawn and diluted, again in a volumetric flask, to 25 cc.

Such solutions gave concentrations suitable for accurate measurement of the two main maxima (at approximately 6630 and 6180 Å) and the minimum between these two. Only occasionally were readings made outside of this range. A few other absorption curves will be discussed to explain the changes noted, which fall into four types, all of which are reflected in differences in the absorption curves of the various extracts.

The changes observed are illustrated in figures 1 to 4, the first three of

which deal with decomposition products of chlorophyll, in the solution, and the curves in each figure were selected to show progressive stages in the development of these products. For convenience only skeletal curves, containing the log E values for wave lengths of major changes, are presented, and the points are connected by straight lines. All curves in this section are considered in relation to the main maximum, for present purposes to be taken at 6650 Å, though it is recognized that absolute values will necessarily differ, especially where there has been partial decomposition. The changes observed are as follows: 1. Pheophytin formation. In figure 1 will be found the effect of increasing transformation of chlorophyll to pheophytin on the shape of the curve. It is seen that absorption is greatly diminished from 6400 to 6100 Å. The second maximum is shifted from 6180 Å to 6070 Å, and a prominent band appears in the green at 5350 Å. Incipient pheophytin formation is well illustrated in figure 1, curve 2, for sorrel (solvent extrac-

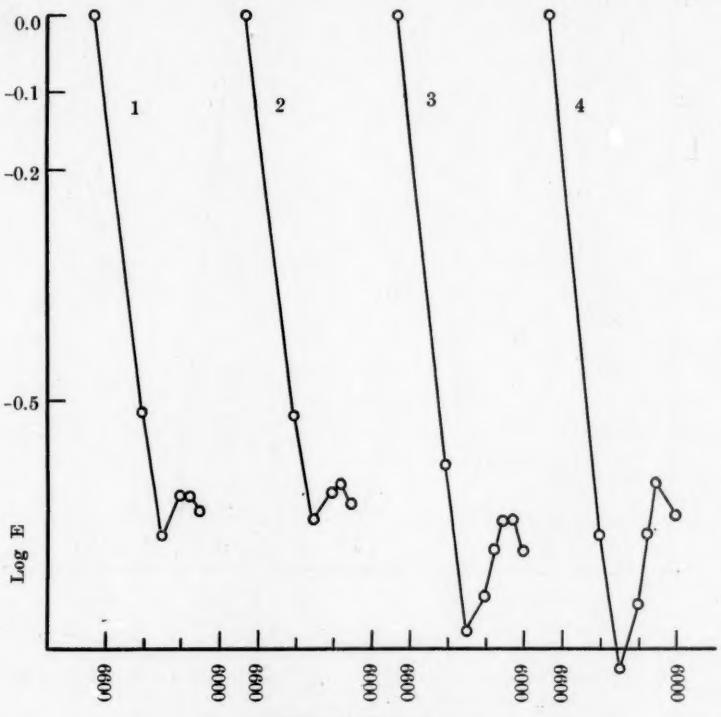

FIG. 1. Stages in pheophytin development. 1, sorrel dried *in vacuo;* 2, sorrel, solvent extraction; 3, sorrel, dipped in boiling water; 4, begonia and oxalic acid.

tion). Pheophytin is much less strongly adsorbed than the chlorophylls, and in fact also less strongly than some of the xanthophyll carotenoids, on inulin, on which it appears as a dark brown zone which, on elution, is olive brown in color.

2. Hydrolytic changes. Spectroscopically this is shown by a marked decrease in absorption at 6400 Å (again relative to the maximum). When leaf extracts are partitioned between petroleum ether and 80 per cent. aqueous acetone, the extent of hydrolysis may be gauged by the proportion of green pigment remaining in the aqueous phase. The effect on the absorption is illustrated in figure 2.

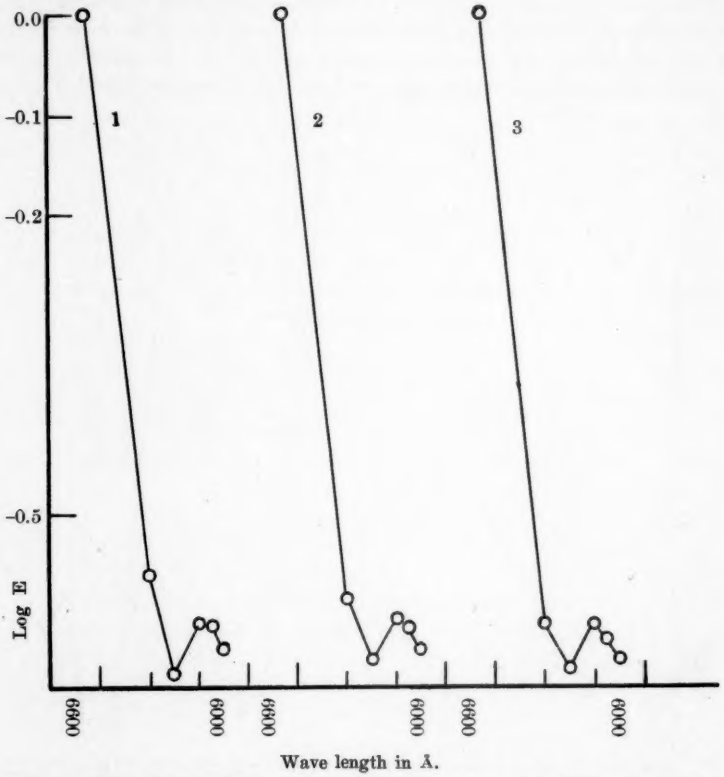

FIG. 2. Stages in hydrolysis. 1, sunflower, dipped in water, 100° C.; 2, sunflower, freezing; 3, sunflower, solvent immersion.

3. Oxidation. An oxidized component is detected in most cases by a decrease in the difference between the two red maxima. The solution has lost its specificity of absorption to some extent. A third component is also

detected on a TSWETT adsorption column. Some confusion may exist at this point, because the position of the oxidized product is not invariably the same, though for a given leaf killed by a given method the result is reproducible. This is reasonably explained on the ground that the oxidized portion has decomposed to varying degrees. Most frequently this portion is associated with the green component, and is held firmly on the column while the latter can be eluted with dichloroethane. It may be pointed out at this juncture that the absorption maxima for these allegedly oxidized components are intermediate between those of chlorophylls *a* and *b*, and that they can be eliminated or held to a fraction of one per cent. of the total pigment by selection of a suitable extraction method. Differences in the curves are shown in figure 3.

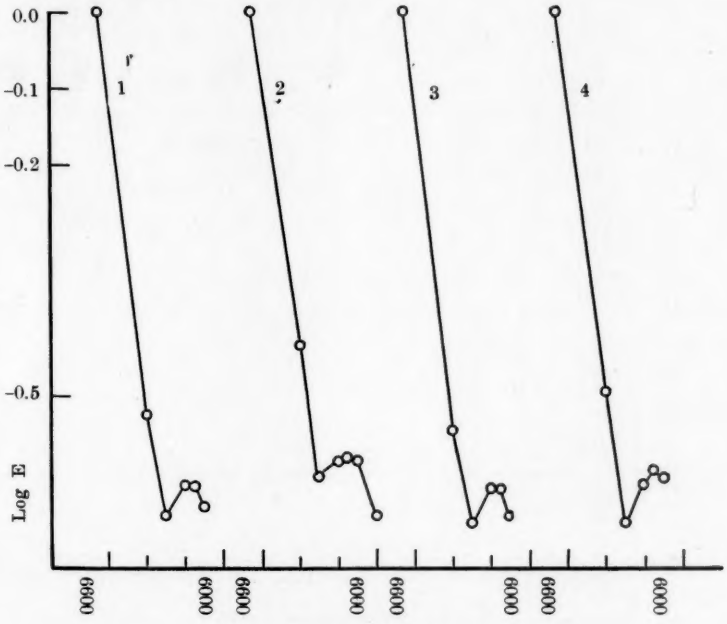

FIG. 3. Effect of oxidation. 1, clover, toluene in oxygen; 2, clover, toluene in air; 3, barley, solvent extraction; 4, barley, toluene in air.

4. Fluctuations in the ratio of chlorophylls *a* and *b*. The effect of modification of these ratios on the shape of the curves is shown in figure 4. Chlorophyll *b* has a maximum absorption near the point where chlorophyll *a* shows a minimum, at approximately 6400 Å. Increase in the proportion of component *b* therefore translates the minimum for *a* substantially toward

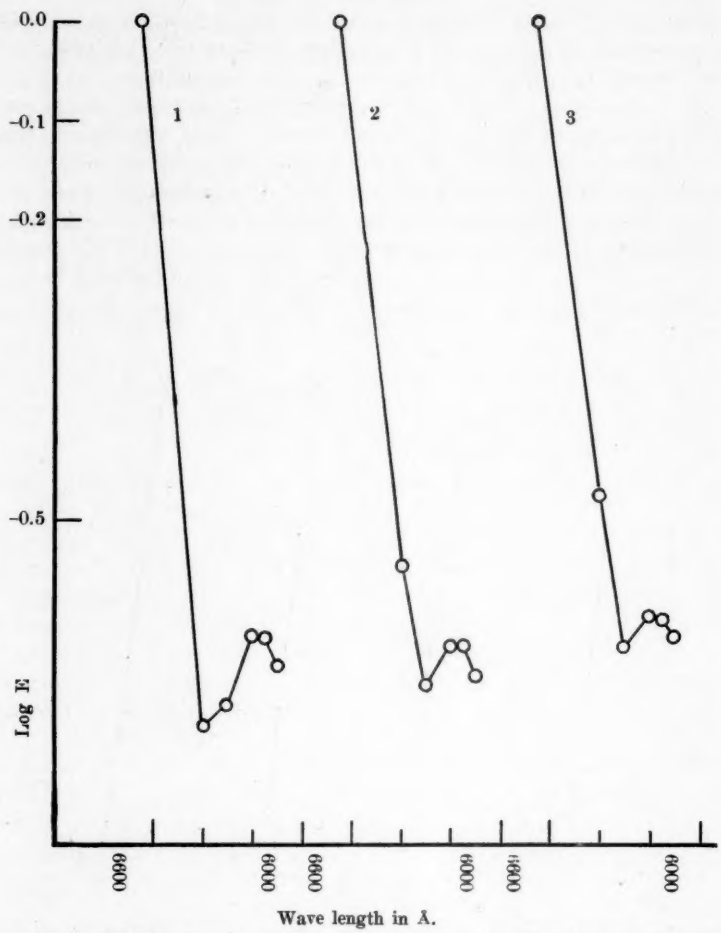

FIG. 4. Variation in ratio of chlorophylls A : B. 1, blue-green algae; 2, begonia; 3, Virginia creeper.

the blue, in the majority of cases from close to 6400 to 6300 Å. The skewness of the main band (chlorophyll *a*, maximum at 6630 Å) is thereby materially increased. The ratio differs substantially for different sources, but, as was found in the compilation of table I, appeared to be so constant for leaves of different ages, collected at different times of the day, that divergences were within the limits reported for the deviations of the mean.

In some cases pheophytin formation and oxidation may occur together, in which case both adsorption and spectroscopic results must be examined.

Changes of the first three types involve degradation or decomposition of chlorophyll. They may be induced by modifications in the methods employed to kill the leaves. In no case have we found evidence connecting fluctuations in the ratio of the *a* and *b* components with the methods employed.

A consideration of figures 1 to 4 indicates the wave lengths to be scrutinized for variations. The eighty-four curves, representing that number of individual plant extracts, have been analyzed by consideration of the constancy of the following differences:

1. $\log E_{6650} - \log E_{6400}$
2. $\log E_{6650} - \log E_{6300}$
3. $\log E_{6650} - \log E_{6150}$

Here E represents $\log_{10} 1/T \times$ constant
whence $\log E = \log \log 1/T +$ constant.

To assess variability within the leaves selected, we summarize in table I the data for apparently unchanged extracts, regardless of method of preparation.

The spectral region most sensitive to change in *b* content is at 6400 Å, near which *a* shows a minimum and *b* a maximum. The plants are listed in table I in order of decreasing *b* content relative to *a* as determined by the values in column 1. The *a* component has a minimum near 6400 Å, the leaf mixture at 6300 Å. A trend in the differences between $\log E_{6650} - \log E_{6400}$ should therefore be followed by a similar trend in $\log E_{6650} - \log E_{6300}$, though on a smaller scale because of the lessened effect of *b* absorption at 6300 Å relative to its absolute value at 6400 Å. This is very satisfactorily demonstrated in column 2 of table I. The last column of table I is substantially, though not entirely, a measure of the difference in the two absorption maxima in the red, of the *a* component. It should therefore be nearly constant. The maximum, in 80 per cent. acetone, is near 6180 Å ($\pm$ 5 Å). Had the values for 6200 Å been chosen, results would have been more satisfactory. It was the writer's practice to re-determine the values for 6650, 6400, and 6150 Å after completing a run, and the values for 6150 Å therefore have a slightly enhanced accuracy, over those for 6200 Å.

The wave length 6150 Å was originally selected because the maximum appeared to be at this point in some of the earlier samples, before the writer was aware that this could be attributed to traces of pheophytin. The exact maxima were not selected at first for two reasons. (1) We had no assurance that slight shifts in the maxima were not caused by our methods of preparation; and (2) for routine examination it was inconvenient to set the wave length drum of the predispersing spectrometer at an unmarked value. Slight variations in this setting caused considerable variation in the amount of

light received by the monochromator, which consequently was reflected in smaller galvanometer deflections.

The Virginia creeper, ivy, and eucalyptus samples were studied mainly for differences in young and old leaves, with respect to the *a: b* ratio. Some sunflower samples were also utilized for this purpose. The Virginia creeper leaves were less than 4 cm. and greater than 12 cm. in length, petioles being discarded. Corresponding values for ivy were less than 5 cm. and greater than 8 cm., representing a year's difference in age. Giant leaves (38×38 cm.) of sunflower, about 5 months old, were compared with young leaves 14 cm. long by 8.5 cm. at the widest point, approximately 3 weeks old.

The time of day for collection of samples varied from 9 A.M. to 4:30 P.M. Eucalyptus leaves were of the broad, sessile, so-called "juvenile" type, both

TABLE I

ANALYSIS OF CURVES FOR ALL METHODS WHERE NO CHLOROPHYLL DEGRADATION PRODUCTS WERE DETECTED

LEAF	NO. OF DETERMINATIONS	$(1)_{6650-6400}$	$(2)_{6650-6300}$*	$(3)_{6650-6150}$
Virginia creeper	5	0.474	0.628	0.600
Eucalyptus	4	0.494	0.642	0.607
Sorrel	3	0.508	0.654	0.609
Ivy	2	0.515	0.659	0.620
Begonia	2	0.531	0.659	0.615
Clover	15	0.532 ± 0.004	$0.657 \pm .006$	0.617 ± 0.006
Barley	5	0.538	0.660	0.615
Sunflower	16	0.558 ± 0.003	$0.667 \pm .004$	0.620 ± 0.002
Tobacco	5	0.561	0.673	0.624
Blue-green alga	1	0.709	0.686	0.615

* These numbers mean log E 6650 – log E 6300, etc. See also tables II, III, and V.

small and large, and also of the long narrow petiolate "adult" type, both young (9 cm. long) and old (24 cm. long). Where the method of extraction was reliable, differences were small. The mean deviations of the mean are reported for sunflower and clover, because of the larger number of samples. The deviations for the other samples are of the same order of magnitude, in no case as high as $\pm$ 0.01 logarithm unit, which would be 2 per cent.

Having ascertained the shapes of curves of unchanged extracts, we can now examine the effects of methods unsuitable for a particular leaf.

ROBEN (3) in an extensive study of the effect of heat and cold on pheophytin formation noted that pheophytin is readily formed in leaves with acid sap when dipped into boiling water.

An instantaneous dipping of clover, tobacco, and sunflower has no per-

ceptible effect. This is not true for barley, begonia, and sorrel. The effect is very slight with barley, but sorrel turns brown immediately, and shows, in its spectrum, and on the inulin column a high proportion of pheophytin.

The effect of hot water, if prolonged, apparently is to bring about this change even in leaves whose sap is normally near neutrality. In table II will be found examples of partial transformation of chlorophyll to pheophytin. This is also illustrated in figure 1, where the effect of adding oxalic acid to a begonia extract may be compared with dipping sorrel into boiling water, a clear example of identity of the changes.

TABLE II

EFFECT OF PHEOPHYTIN FORMATION ON ABSORPTION

LEAF	METHOD	$(1)_{6650-6400}$	$(2)_{6650-6300}$	$(3)_{6650-6150}$
Clover	Dip in boiling water	0.531	0.646	0.605
	5 minutes in boiling water	0.542	0.679	0.622*
Sunflower	Dip in boiling water	0.563	0.663	0.621
	5 minutes in boiling water	0.577	0.683	0.635*
Barley	Dip in boiling water	0.550	0.681	0.634*
Sorrel	Dip in boiling water	0.575	0.800	0.684†
Begonia	+ oxalic acid	0.673	0.842	0.673

* Incipient pheophytin formation. Band at 5350 Å is definitely perceptible.

† Sorrel and begonia have different a/b ratios, hence only general trends in the two cases are to be noted.

The values noted in table II should be compared with corresponding values for "normal" extracts in table I.

Solvent extraction is in nearly all cases satisfactory, if rapidly performed. If the plant is extremely acid, drying *in vacuo* is sometimes preferable. With sunflower leaves, in two instances, where samples were collected, plunged into acetone, and kept in the dark for an hour prior to extraction, hydrolysis occurred. The sap of the photosynthetic tissue was sufficiently alkaline to cause saponification. This is apparently exceptional, and can be obviated by immediate extraction. It would appear, from the work of FIFE and FRAMPTON (1), to cite but one example, that the CO_2 in the leaf has a marked effect on the pH, and its removal would cause the sap to revert

to a more nearly neutral condition less harmful to the chlorophyll. Hydrolytic changes have been noted in figure 2.

Drying *in vacuo,* at room temperature, or in air at 45–56° C. yields good results, if the leaf does not have a waxy cuticle or is covered with minute hairs, or has a high content of reserve carbohydrates. Tobacco leaves, whether dried *in vacuo* or at elevated temperatures, gave evidence of oxidized components differing from those of other leaves chiefly in the lower position they occupied on the inulin adsorption column, relative to components *a* and *b*.

Leaves toluenized and subsequently dried in an atmosphere of hydrogen gave satisfactory extracts, but only sunflower leaves could be toluenized in air without the occurrence of oxidation and, in many cases, also pheophytin formation.

Some results with toluene and chloroform are given in table III. The chloroform was washed, dried with anhydrous potassium carbonate, and used within 5 minutes of re-distillation. Changes are apparently similar to those with toluene.

TABLE III

EFFECT OF OXIDATIVE CHANGES ON ABSORPTION CURVES

LEAF	METHOD	$(1)_{6650-6400}$	$(2)_{6650-6300}$	$(3)_{6650-6150}$
Clover	Toluene in air*	0.466	0.685	0.612
	Toluene in air	0.437	0.607	0.580
Barley	Toluene in air	0.518	0.669	0.595
	Chloroform in air	0.516	0.643	0.601
Tobacco	Dry *in vacuo**	0.584	0.691	0.643
	Dry 45–50° C.*	0.590	0.699	0.667

* These are obscured by pheophytin formation, which tends to counteract, spectroscopically, the effect of oxidized components.

Samples from a given collection were usually killed simultaneously by at least two methods. In cases where fresh weights were accurately obtained (the leaf loses weight with great ease) comparisons of the "E" values for a given wave length became possible. Where the method has no deleterious effect, E is constant within a sampling error of ± 5 per cent. Where changes have taken place, E may fall as much as 50 per cent., indicating either a substantial loss of pigment, or the formation of pigments with proportionately lower capacity for absorption. A few results are given in table IV.

Conclusions

The method of killing a leaf preparatory to making a pigment extraction may significantly modify the chlorophyll pigments. This is of fundamental

TABLE IV

EFFECT OF METHOD ON E_{6650}

LEAF	METHOD	E_{6650}	REMARKS
Clover	1. Toluene in air	0.335	Oxidation
	Dried in air	0.612	
	Dried in H_2	0.623	
	2. Toluene in H_2	0.245	
	Toluene in air	0.184	Oxidation
Sunflower	1. Toluene in air	0.847	
	Dried *in vacuo*	0.880	
	Dried in 45–50° C.	0.801	
Tobacco	1. Dip in boiling water	0.608	
	Freeze	0.620	
	2. Solvent extraction	0.414	
	Dried 45–50° C.	0.300	Oxidation and pheophytin
Barley	1. Solvent extraction	0.399	
	Dried *in vacuo*	0.365	
	2. Dried 45–50° C.	0.389	
	Dried *in vacuo*	0.389	
	Toluene in air	0.305	Oxidation

import in studies purporting to discuss fluctuations in the *a* and *b* components of chlorophyll, because traces of pheophytin and other degradation products will profoundly modify the picture presented by spectroscopic data. The greatest danger arises with plants whose sap is decidedly acidic. Anaesthetics, by modifying the phase relationships in the pigment-containing bodies, thereby exposing the pigments to the air, may have an equally serious effect on plants whose sap is nearer neutrality. They should be used therefore only in the presence of an inert gas.

It is of interest that the ratio of chlorophyll *a* to chlorophyll *b* apparently fluctuates within rather narrow limits, regardless of age of tissue or time of day. It is to be understood that the constancy of this ratio applies only to these two factors for normal healthy leaves and that no conclusions may be inferred for etiolated or chlorotic leaves nor for environmental factors widely differing from those obtaining in the field.

Substantial differences, however, are recorded for different plants. Of minor interest is the identity of pigment ratios in the juvenile and adult types of leaves of the eucalyptus.

PART II

SEPARATION OF BLUE AND GREEN COMPONENTS BY ADSORPTION

It is necessary to examine further the criteria set up to determine that

there has been no fundamental change in the chlorophylls of clover, for example, whether killed by dipping in boiling water, by solvent extraction, or by drying. There is the substantially identical nature of the absorption spectrum curve, but this differs significantly from corresponding curves for sunflower or tobacco, and it becomes necessary to inquire to what extent we are justified in ascribing these differences solely to fluctuation in the ratio of the two chlorophyll components.

The simplest method of answering these questions is to show that the unaltered extracts contain only two chlorophyll components which have identical absorption spectra regardless of the source of material. Until we have devised preparative methods for isolation in pure solid form of the two components we are not justified in assuming the blue component to be unaltered chlorophyll *a*, nor the green, unaltered chlorophyll *b*. We have, however, been able to show that the leaves studied give blue and green components of whose spectroscopic identity there can be no reasonable doubt.

When it becomes possible to show that our blue and green components are unaltered chlorophylls *a* and *b*, we may hope to give a definite answer to the problems raised by the papers by WINTERSTEIN and STEIN (**7**), WINTERSTEIN and SCHÖN (**6**), and by ZSCHEILE (**8**). No contribution can be made to the subject which tacitly ignores or refers only by implication to the differences of opinion. Furthermore, the fact must be bluntly stated that no chlorophyll absorption curves have been published which are accepted unreservedly by workers in this field.

The evidence presented in this section is definitely unfavorable to ZSCHEILE's suggestion of a third component of chlorophyll. If it be pointed out that his methods of extraction were not dissimilar to those found to be satisfactory in this paper, it need hardly be emphasized that there is inevitable difficulty in working with 1 or 2 kg. of fresh material, not encountered when 2 to 5 gm. are worked up in as many minutes, for rapid spectroscopic analysis. The writer considers it inevitable that chlorophyll prepared by the method of WILLSTÄTTER and STOLL must contain some quantity of an oxidized component, the extent of oxidation depending no doubt upon the leaf material used and upon the skill of the worker. As already pointed out, the separations by adsorption are not quantitative. Thus WINTERSTEIN and STEIN (*loc. cit.*, p. 274) from 3 gm. chlorophyll (60 per cent. *a* : 40 per cent *b*) obtain 700 mg. of *a*, and 1300 mg. *b*, with 300 mg. of mixture.

Difficulties of this type have undoubtedly given rise to the search for other methods and a corresponding neglect of the adsorption technique, particularly in its most recent developments. Thus MILLER (**2**) writes: "Recently ZSCHEILE (**11**)* and MILLER (**7**) have shown that the spectrophotoelectric method satisfactorily solves the problem of pigment differentiation.

* Numbers here refer to MILLER'S citations, *q.v.*

This method makes possible not only the determination of the total differentiation for each group (chlorophylls, carotenes, and xanthophylls), but also the analysis for the total of each component within the respective groups. Because no separation of pigments into groups or components within a group is necessary, the spectrophotoelectric method is rapid."

With this, the writer is in profound disagreement. Where spectroscopic data indicate significant changes in the pigment content, supplementary evidence must be obtained to avoid the ascribing of such changes to erroneous causes. Furthermore, BEER's law cannot as yet in practice be extended to cover a dozen or more xanthophyll components, 4 or 5 hydrocarbon carotenoids, 2 (or 3) chlorophyll components, together with such modifications of the chlorophylls as may appear in a study of methodology, without "separation of pigments or components within a group." In no way now known can the necessary evidence be more easily obtained than by the TSWETT adsorption method.

In considering next discrepancies in the absorption spectra of the two recognized components, we come to an extremely unsatisfactory stage. In proceeding with detailed criticism, we must admit certain limitations. As already stated, a definitive contribution requires that we isolate, in solid form, spectroscopically pure chlorophylls *a* and *b*. Our only claim for inulin as yet is that we are enabled to obtain rapidly blue and green components in solution, free from other pigments. The detailed observations of ZSCHEILE and the numerous analyses by WINTERSTEIN and STEIN are evidence of the magnitude of the task confronting anyone who would make this contribution, though we hope the inulin procedure may offer a means of obviating the long and laborious method now in use for the isolation of chlorophyll. (For a goal which requires spectroscopically pure components, the method of WILLSTÄTTER and STOLL may be dangerously long.)

Our criticisms are offered at this juncture because it is now four years since WINTERSTEIN and SCHÖN replied to ZSCHEILE's paper, during which period there has been a great increase in the hypotheses propounded and solutions offered as to the photosynthetic mechanism, and withal, few realize that no adequate absorption coefficients have been reported, which as we have stated, are unreservedly acceptable.

We find ZSCHEILE in agreement with WINTERSTEIN and STEIN that chlorophyll *b* prepared by the method of WILLSTÄTTER and STOLL may contain a considerable percentage (15–20) of component *a*. There can be little justification for the comparison by WINTERSTEIN and SCHÖN (*loc. cit.*, p. 140) of their results in benzene with ZSCHEILE's values in ether. The apparent reversal in the relative heights of the maxima in ether solution, at 663 mμ and at 432 mμ (*WINTERSTEIN and STEIN, loc. cit.*, table, p. 270), remarked by ZSCHEILE, is only explained later (**6**, p. 141) as a subjective observation.

However, the presence of two absorption maxima, at 623 and 607 mμ for the *a* component in ether, and at 614 and 594 mμ for *b* (*loc. cit.*, p. 268), which are certainly not all represented in the absorption curves for the components in benzene solution (fig. 1, p. 268), indicates either that solvent differences cannot be ignored, or that ether may be a particularly unreliable solvent owing to the ease of peroxide formation. The meaning of the statement by WINTERSTEIN and STEIN (*loc. cit.*, p. 269): "Es ist möglich dass die beiden für Chlorophyll *a* bei 623 und 607 mμ sowie die beiden bei 507 und 494 mμ angegebenen Doppelbänden doch nur Einzelbanden sind," is not clear to the writer except insofar as the observations are still subjective.

Measurements reported in this paper, though on a relative scale, may be compared with the results of WINTERSTEIN and STEIN, and of ZSCHEILE. No serious disagreement is found, either in dichloroethane or in 80 per cent. acetone, with ZSCHEILE's values, measured objectively in ether, nor with those of WINTERSTEIN and STEIN, in benzene. The solvent has apparently very little effect on the positions of the maxima—contrast in this respect, the positions of the maxima for β-carotene in carbon bisulphide and in ethanol (**4**)—nor yet on the differences in the absorption coefficients between the maxima. We have as yet no criterion for comparing the absolute values, where WINTERSTEIN and STEIN and ZSCHEILE are in most serious disagreement.

We cannot determine precisely how WINTERSTEIN and SCHÖN (*loc. cit.*, p. 140), re-calculated ZSCHEILE's values. If we take ZSCHEILE's value of 91.3, the highest reported by him for component *b*, at 4525 Å, this should be multiplied by the molecular weight, approximately 910, and by 2.303 to convert the specific coefficient (logarithm to base 10) to the molar coefficient (logarithm to base e). Thus $91.3 \times 910 \times 2.303 = 191 \times 10^3$, not 236.9×10^3, as reported by WINTERSTEIN and SCHÖN.

For effective separation of the components on inulin we found it necessary to introduce another solvent, dichloroethane. There is but little displacement of the band maxima in this solvent as compared with ether. As the values reported here are on a relative scale, it is possible to compare our blue component with their *a*, and the green with *b*. No direct comparison can be made of blue and green components.

It is of interest that differences in the maxima correspond quite closely with those reported by ZSCHEILE, and we agree further with him in finding a minimum for the green component at 6150 Å (ZSCHEILE, with ether gives this at 6140 Å).

Experimentation

The following leaves were studied: clover, sunflower, tobacco, barley, sorrel, Virginia creeper, and ivy.

Two to five gm. of leaf material, prepared by a method suitable for each

particular leaf, were extracted with 50 cc. of 80 per cent. acetone, transferred to 15 cc. of petroleum ether which was then washed and dried. Five to ten cc. of this solution was adsorbed on a column of inulin, diameter 2 cm., length 5 to 10 cm. This was washed exhaustively with benzene-petroleum ether (b. p. 55–65° C.), 3 : 1 by volume, until all carotenoids had been eluted. This required 150 to 200 cc. of solvent. Redistilled washed dichloroethane was then added to effect a rapid separation of blue and green components. The first fraction eluted was blue. Where no oxidation took place, the green component was also completely removable with dichloroethane. A third component, found where undesirable extraction methods had been used, was held very strongly as a green or greenish-gray band, and eluted with acetone or ethanol.

Spectroscopically, the third component was intermediate between the pure green and the pure blue components. Both green and blue eluates should be passed over a second column to remove traces of the other, though this was not done in all cases because both components tend to bleach on prolonged exposure to light, and immediate observations were desirable. The solutions at 0° C. in the dark keep their color apparently unimpaired, at least three weeks.

In no case could differences in wave length of maximal or minimal absorption be tolerated, but differences of 0.02 to 0.03 unit in log E, and deviations in the general shape of the curves were permitted where it was reasonably certain they could be eliminated by more rigorous purification.

The essential points to be noted here are, first, the ease with which solutions of the individual components could be prepared by adsorption on inulin, in some cases within three hours from picking the leaves; and second, an agreement in their absorption spectra.

Study was confined to the regions 6700 to 6000 Å, and 4800 to 4000 Å, where the most critical differences between the blue and green components are known to exist. The solvent used was dichloroethane. The blue component in all cases gave maxima at 6630 and 6180 Å in the red, with a minimum at 6380 Å. In the blue, a maximum was found at 4300 Å, and a second small maximum, sometimes only a shelf, at 4150 Å.

The green component gave only one maximum in the red, at 6460 Å, with a minimum at 6150 Å. If another band exists between 6200 and 6000 Å, it is of the order of 0.005 log unit or less above the neighboring points. In the blue region, maxima were found at 4600 and 4350 Å.

In table V will be found the following criteria for determining the constancy of the shape of the curves for the respective components. For the blue component, these are the differences in the coefficients for the respective maxima at the wave lengths noted:

TABLE V

DIFFERENCES IN LOG E FOR VARIOUS MAXIMAL WAVE LENGTHS

BLUE COMPONENT

LEAF	$(1)_{4300-6650}$	$(2)_{4300-4150}$	$(3)_{6650-6200}$
Sunflower	0.106	0.125	0.693
Barley	0.112	0.121	0.667
Clover	0.112	0.120	0.675
Sorrel	0.150*	0.122	0.698
Tobacco	.....†	.....†	0.690
Virginia creeper	0.112	0.127	0.675
Ivy	0.104	0.125	0.666

* Contained traces of carotenoid. Speed was essential because of the high acidity of this plant.

† Contained traces of green component discovered by readsorption.

GREEN COMPONENT

LEAF	$(1)_{4600-6450}$	$(2)_{4600-4350}$*
Sunflower	0.437	0.265
Barley	0.445	0.378
Clover	0.451	0.377
Tobacco	0.459	.....†
Sorrel	0.448	0.302
Virginia creeper	0.433	0.366
Ivy	0.424	0.264

* The band at 4350 was sometimes poorly defined, where it appeared a shelf. This is the only really unsatisfactory group in the table, its proximity to the blue maximum at 4300 Å makes it suspect.

† Contained traces of blue component.

1) $\log E_{4300} - \log E_{6650}$
2) $\log E_{4300} - \log E_{4150}$
3) $\log E_{6650} - \log E_{6200}$

and similarly for the green component:

1) $\log E_{4600} - \log E_{6450}$
2) $\log E_{4600} - \log E_{4350}$

The constancy of the differences listed in table V is extremely satisfactory, particularly for the differences between the two main maxima for each component in the blue and red regions of the spectrum. It is, indeed, rather striking evidence of the efficacy of inulin as an adsorbent, that such a relatively high degree of spectroscopic purity could be attained, in the majority of cases, by the use of a single column of adsorbent, without recourse to further purification by this means, beneficial though this might well have

been. Two other factors may be briefly considered. First, the most intensive study was devoted to the main absorption maxima for each component. Hence the concentrations of the solutions were made most favorable for their measurement; and second, dichloroethane, and presumably therefore this applies to all less stable chlorinated solvents, is far from an ideal solvent with respect to stability of the components, if exposed to light, though it was most satisfactory in the separation and elution of the components on the inulin column.

Finally it should be recalled that in no case was there any difference in the positions of maxima and minima on the wave length ordinate.

A critique of one very recent paper (RABINOWITCH, E., and WEISS, J. Reversible oxidation of chlorophyll. Proc. Roy. Soc. London A **162**: 251–267. 1937) cannot in fairness be attempted here.

These authors consider the differences in band maxima (most evident in the case of chlorophyll *b*) between their results and ZSCHEILE'S, may be explained in part on the basis of HUBERT'S results in different solvents (HUBERT, B. Estimation of the band position of chlorophyll in different media. Proc. Kon. Akad, Wetensch. Amsterdam **37**: 3–8. 1934). Apparently HUBERT used leaf chlorophyll, and his data for ether and acetone extracts are the reverse of those now under consideration, though the difference he reports for these solvents is slight, of the order of 15 Å. The greatest uncertainty lies in the spectroscopic characterization of chlorophyll *b*, and the observations of RABINOWITCH and WEISS strengthen the writer's contention that extreme caution must be used in evaluating spectroscopic data (in many instances of high precision) in terms of possible biological significance.

Conclusions

We feel justified in considering the leaves studied, when extracted by appropriate means, to contain only two chlorophyll components, spectroscopically identical with similar components from different leaf sources. When therefore leaf extracts show differences in their absorption spectra which cannot be ascribed to method of leaf-killing, it may be concluded there are differences in the ratio of the two chlorophyll components. Adsorption studies on inulin are definitely unfavorable to ZSCHEILE'S hypothesis of a third component, in which respect we agree with WINTERSTEIN and SCHÖN. It has been pointed out that a definitive answer is not yet possible on the absorption coefficients of the pure chlorophyll components. Our results in dichloroethane are in better accord with ZSCHEILE'S interpretations of his own results than with those of WINTERSTEIN and SCHÖN. A discussion of the differences has also been presented.

The writer is indebted to Dr. H. A. SPOEHR and to Dr. JAMES H. C. SMITH for their active assistance, suggestions, and criticisms.

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EFFECT OF LIGHT ON CO_2 IN LEAVES

JOHN SHAFER, JR.[1]

(WITH THREE FIGURES)

Introduction

Recently, in an attempt to find some correlation between the average stomatal aperture of a leaf and the amount of carbon dioxide in that leaf, the author developed a piece of apparatus with which to extract and analyze the gas in a single leaf. In time it became apparent that no correlation between stomatal aperture and carbon dioxide was being found. At about the same time it began to appear that darkness is associated with a decrease in the amount of carbon dioxide in leaves. Even before this it had become obvious that leaves contain large amounts of carbon dioxide when they are in bright light. The latter part of the research was turned to the apparently new study of light effect on the carbon dioxide contents of leaves.

The extraction of gases from the leaf was performed essentially in the way followed by MAGNESS (**6**). The analysis of gases obtained followed the basic principles employed in any of several common volumetric gas analysis apparatuses. The analysis part of the apparatus approaches most closely, perhaps, that of BONNIER and MANGIN (**2**, p. 377).

Most of the work leading to the data in this paper was done on the broad bean, *Vicia faba* L. This was a very satisfactory plant because of its opposite, glabrous leaves. The following plants also were used at one time or another: nasturtium, *Tropaeolum majus* L.; beet, *Beta vulgaris* L.; wandering Jew, *Zebrina pendula* Schnizl.; red kidney bean, *Phaseolus vulgaris* L.; a variegated geranium, *Pelargonium hortorum* Bailey; a willow, *Salix* sp.; two varieties of roses, *Rosa* spp.—a white-leaved kind, "Silver Wedding Rose" (furnished by the courtesy of the Albert F. Amling Co., of Maywood, Illinois), and a green-leaved variety, "Senior."

The present research has been based on determinations of the amounts of carbon dioxide which could be extracted from leaves by a Torricellian vacuum. Nearly always the leaves have been used singly, each determination giving the amount of carbon dioxide in the gases obtained from one leaf (or leaflet, in the case of *Vicia*).

Apparatus

The samples have been extracted and analyzed in the apparatus shown in figure 1. The flask Q holds an aqueous solution of 30 per cent. sodium hydroxide. Before the apparatus is used, the sodium hydroxide is drawn through the capillary tube *P*, of 1.5-mm. bore, and the stopcock *M*, of 1-mm.

[1] The writer held a Cramer Fellowship from Dartmouth College during this research.

bore; then the stopcock is closed. This arm ($N,P,Q,$) of the system is to be used in the analysis and must be completely filled with sodium hydroxide. Tubes *P* and *N* are joined, the end of each having been ground flat, by a short rubber tube.

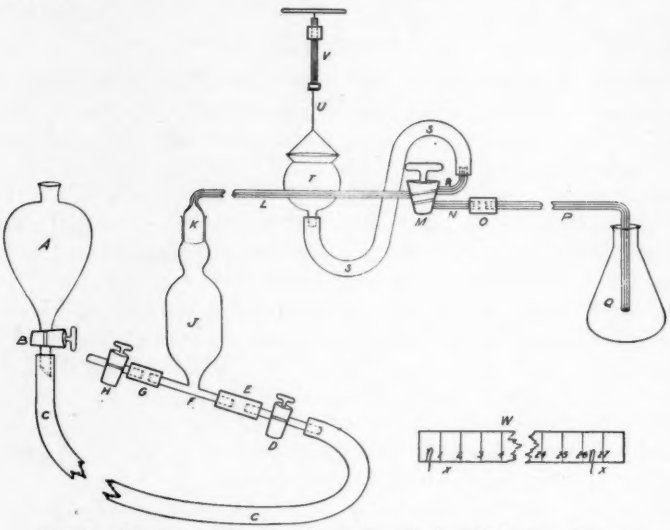

FIG. 1. Apparatus for extraction and analysis of gas from leaves.

Now the capillary tube *L*, of 1.5-mm. bore, must be washed and dried. This tube must be washed frequently. It is easily done by separating the ground glass joint *K* and applying suction to that end of tube *L*. Water can then be drawn through tubes *L* and *S*, followed by alcohol and then by air until the tube *L* is dry.

The entire apparatus, save only the arm *N,P,Q,* must be dry when used. With care all water can be kept from passing joint *K* into chamber *J*; hence this need never be dried. The apparatus is next filled with mercury from the mercury reservoir *A* to the stopcock *M*. This stopcock is closed and the reservoir *A* lowered to produce a Torricellian vacuum. Rubber tube *C* must be nearly 1 meter long. Several successive evacuations are needed to free this part of the apparatus of air. All air must be removed from tube system *G,H,D,C*. It has been impossible to reduce the air below 0.5 cm. (Throughout this paper gas measurements are expressed in centimeters, having been measured always at atmospheric pressure).

Once freed of air, the apparatus is ready to use. The joint *K* is separated and the mercury allowed to drain partially from chamber *J*. Chamber *J* is completely emptied only when under vacuum; only in this way can air

be kept from going below cross-tube *F*. Into the partly empty chamber is introduced a leaf. Then the joint *K* is quickly closed and mercury is driven in to fill chamber *J*, tubes *L* and *S*, and half of levelling tube *T*. Stopcock *M* is then closed and the reservoir *A* lowered. As the chamber *J* empties, various gases quickly emerge from the leaf. After half a minute or so the mercury is returned to chamber *J*, driving the gases extracted from the leaf into tube *L*. No more attention need be given to the leaf; it will not give off any gas so long as it is covered by mercury at atmospheric pressure, and even if some were produced, it could not mix with the sample being analyzed.

For analysis the gas must be measured at some constant pressure. Atmospheric pressure is the easiest pressure to attain and is reasonably constant. Stopcock *D* is closed and that at *M* opened. The pressure is then adjusted by screwing the levelling bulb *T* up or down. The scale *W* is at this time hooked onto tube *L*. The scale used in the present work was 28 cm. long and was divided into millimeters. With a hand lens of about 7× power one can read the mercury meniscus to tenths of millimeters with an accuracy of ± 0.01 cm. The gas bubble is most easily measured by taking the difference between the readings of its two ends.

Once the length of the bubble is known, most of the mercury between the bubble and the 3-way stopcock *M* is forced into the tube system *S*–*T*. Then the stopcock is reversed, and the rest of the mercury and all of the gases are allowed to pass into the tube system *N*–*Q*. It is best to stop in the stopcock *M* the mercury following the gas. The presence of the mercury globule in front of the gas bubble has several advantages and is quite necessary. It leaves in the tubes *N* and *P*, however, enough hydroxide solution to absorb all of any bubble introduced into those tubes, even if the bubble is pure carbon dioxide. This has been determined experimentally. Experiment has also shown that all of the carbon dioxide is removed from a sample within thirty seconds after the last of that sample has entered the hydroxide-holding tube. Therefore, at the end of half a minute the gas is drawn back into tube *L* and its length determined as before. The difference between the former and this latter bubble length is, of course, a measure of the carbon dioxide originally present. This second reading completes a determination. Several centimeters of the hydroxide solution are drawn from tubes *N* and *P* to remove mercury and exhausted sodium hydroxide. Finally the system from *K* to *T* is washed and dried, preparatory for another analysis.

Certain experimental difficulties which have been met are these: (1) Some parts of the apparatus (especially the stopcock *M*) may trap part of the sample. (2) The leaf may in one way or another trap large amounts of air, thus making the sample so large that part of it must be discarded. (3) Volume changes of the sample may be large—changes due to pressure and room temperature are slow and can be disregarded as regards single analyses. The

greater variations due to these causes over a period of days will tend to equalize each other. The proximity of the operator's body to the apparatus will cause rapid temperature, and therefore volume, changes. (4) Occasionally tube *L* and stopcock *M* become so dirty that they must be cleaned; this is easily done with aqua regia. (5) After a period of use, the stopcock *M* allows some of the sodium hydroxide solution to pass and must be greased again. (6) Some leaves, *e.g.*, those of *Vicia*, take mercury into their intercellular spaces, thereby causing the gas bubble to move toward the chamber *J*.

Various experiments to test the accuracy of this gas analysis indicate that the maximum error for any one reading is $\pm$ 0.10 cm. Only rarely does the error for any one reading exceed this value.

The bore of capillary tube *L* must be nearly constant, if one is to get accurate readings. The bore of the tube used in the present work was tested in this way: a globule of mercury about 1 cm. long was introduced into the tube and measured between each two adjacent centimeter marks on the scale from one end of the tube to the other. This was repeated ten times. Then the ten readings for each centimeter region were grouped and averaged. These average values were used in constructing the graph of figure 2. The value of σ was calculated for each of the average values:[2] the highest σ was 0.0217, the lowest 0.0159, the average 0.0183. Note in figure 2 that a mean

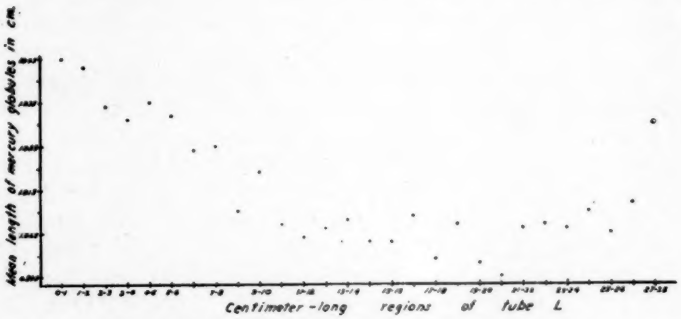

FIG. 2. Graph showing variations in capillary tube *L*.

value of 1.005 $\pm$ 0.10 cm. includes most of the length of the capillary tube. The ordinate values in the graph mean nothing as absolute lengths, but they undoubtedly show the relative cross-sectional areas of the different regions of the tube with considerable accuracy.

Whether the bore of tube *L* is constant enough for use must finally be determined by the accuracy of the readings which are obtained in actual analyses. The work reported in this paper can at best have been only semi-

[2] Thanks are due to Professor J. R. LIVERMORE for help with the statistical part of the problem.

quantitative. Therefore, the accuracy of the apparatus may be considered sufficient if the errors do not completely obscure the results.

Methods of analysis

Two methods have been used to test the working accuracy of the apparatus. The one employed the analysis of samples of carbon dioxide-enriched air. The other employed the analysis of numerous samples of atmospheric air taken from the laboratory.

The results of the tests employing carbon dioxide-enriched air are summarized statistically in table I. The method used was to enclose in a flask

TABLE I

STATISTICAL SUMMARY OF DATA FROM ANALYSES OF CO_2-ENRICHED AIR*

DATE	AV. CO_2	N	σ_M	ERROR OF SINGLE OBSERVATION	$\% \times N$
				%	
3/16/36	0.40	15	0.102	25.4	381.0
3/17/36	0.30	15	0.082	27.3	409.5
3/19/36	0.33	10	0.065	19.7	197.0
3/20/36	0.31	10	0.030	9.7	97.0
					50/ 1084.5
					21.7

* On the basis of the 50 observations included in this summary, the error of a single observation is 21.7 per cent.

the enriched air and to invert the flask over one arm of a U-tube. The mouth of the flask was sealed with mercury. The free end of the U-tube was attached to stopcock *H* of the apparatus. A sample could be drawn at will by evacuating chamber *J*, then opening stopcock *H*. Ten or fifteen small samples were drawn from the flask before it had to be refilled. It was found that the gas in the flask varied in composition unless the mercury within and outside of the flask was maintained at nearly identical levels. This was done in the tests.

The accuracy of analysis was tested in another way from these same data; the average percentage of carbon dioxide in each flask was calculated from the analytical data for the various small samples. From the average percentage it was possible to calculate what carbon dioxide reading should have been obtained from each individual analysis. This assumed that the average carbon dioxide value for a flask was a correct measure of the carbon dioxide concentration. The difference between any calculated value for carbon dioxide and the corresponding experimentally determined value was taken to be

experimental error. Results falling within $\pm$ 0.10 cm. of the calculated value were obtained from 42 of the 50 analyses.

For the tests using atmospheric air, the samples were drawn directly from the laboratory air. If the concentration of carbon dioxide in that air had been 0.03 per cent., the maximum reading obtained for carbon dioxide should have been 0.01 cm. Since the error in reading each mercury meniscus was by itself 0.01 cm., ordinary air has been considered for present purposes to have no carbon dioxide. Any difference in bubble length has been assumed to be experimental error. Of the 60 analyses made, 51 were included within limits of 0 $\pm$ 0.1 cm. (table II).

Data from the results of the analyses of moist and dry air are also included in table II. The general technique used was that employed in obtaining samples of carbon dioxide-enriched air. Dry air was prepared by having in the flask for several days small chunks of potassium hydroxide. Moist air was prepared by having water in the flask for several days.

TABLE II

RESULTS OF ANALYSES OF AIR TO SHOW VARIATIONS DUE TO RELATIVE HUMIDITY

CONDITION OF AIR USED	AV. TOTAL VOLUME	AV. "CO_2" READINGS $\pm \sigma_M$	CO_2	NO. OF ANALYSES	NO. OF READINGS IN LIMITS $\pm$ 0.1 CM.
			%		
Untreated	23.681	0.021 $\pm$ 0.012	0.087	60	51
Dried over KOH	22.409	0.019 $\pm$ 0.014	0.086	15	13
Moist over H_2O	20.809	0.010 $\pm$ 0.009	0.046	29	28

Of the data for dry air, limits of 0 $\pm$ 0.1 cm. include 13 of the 15 readings. This is equivalent to 52 of 60 readings, and compares well with the 51 of 60 readings obtained by the application of the same limits to the data concerning ordinary air. Of the data for moist air, the same limits include 28 of 29 readings, equivalent to 56 of 60. This, too, is close to the figure obtained from the data for ordinary air.

The apparent complete absence of any effect of water vapor on carbon dioxide readings is extraordinary. Assuming a pressure of 760 mm., a temperature of 20° C., and a 10 M. (40 per cent.) sodium hydroxide solution 70 per cent. ionized, one can calculate that a 28-cm. sample, with no carbon dioxide but with 100 per cent. relative humidity, should show a decrease after absorption of 0.17 cm. This is above the limit of error for an individual reading and should affect the results of a series of readings; yet it seems to have had no effect. Perhaps the methods of changing the humidity of the air were not effective.

Even if the presence of water vapor does affect individual readings, it will not change any final conclusions because all of the present research has been done on leaves; and the amounts of water vapor given off by the various leaves, though it may vary, is without doubt so nearly constant that any error due to the absorption of water vapor by the sodium hydroxide will appear uniformly in all analyses. The relative effect of such an error will, therefore, be zero.

In summary, it seems that the apparatus used in the present research was accurate within limits of $\pm$ 0.1 cm. for any single reading, or perhaps less, if the statistically obtained error of 21.7 per cent. is correct (table I). A summary of all the analyses of carbon dioxide-enriched air, ordinary air, dry air, and moist air shows that limits of $\pm$ 0.1 cm. include 134 of 154 analyses, or 87 per cent. of the total. The accuracy of the average of a series of readings is undoubtedly greater than $\pm$ 0.10; it may be greater than $\pm$ 0.02, to judge from the various σ_M's which have been calculated. The readings are probably unaffected by any variation in the amount of water vapor (table II).

Experimentation

Throughout the work it has been obvious that illuminated leaves contain much carbon dioxide. Experiment 1 of table IV shows data from the analyses of pairs of *Vicia* leaflets and will illustrate the point. Each member of every pair was in light and the two analyses were made in rapid succession. The average amount of carbon dioxide in the first leaflets of the various pairs was 0.322 cm., or 1.53 per cent. of the average total volume of the samples. The value for the second leaflets of the pairs checks well with this, being 0.323 cm. of carbon dioxide on the average, or 1.51 per cent. of the average total volume. Since the gas samples were contaminated by air which had been adsorbed by the surfaces of leaves and apparatus, and perhaps also by air from small leaks, and since these contaminations increased the volumes of the samples without affecting measurably the amounts of carbon dioxide in them, the percentages just given are not percentages of carbon dioxide in the gases actually obtained from the leaves. The percentages in

TABLE III

AVERAGE VALUES OF CO_2 OBTAINED ON 2/27/36–2/28/36

PLANT	DAY		NIGHT	
	cm.	%	*cm.*	%
Tropaeolum	0.31	1.35	0.28	1.20
Vicia	0.35	2.04	0.22	1.26
Beta	0.70	2.89	0.48	2.00

the leaves must have been even higher than these figures indicate. In any case it is to be seen from these data that the vacuum method used draws much carbon dioxide from leaves, even when they are brightly lighted.

From table III it appears that there is not more extractable carbon dioxide in leaves at night than in the daytime. The data for this table were collected over a period of twenty-four consecutive hours and should therefore be comparable. Data obtained after daybreak on 2/28/36 are not included in this summary, for it is possible that the leaves must be exposed to light for a period of time before they gain more carbon dioxide.

These data suggest, indeed, that leaves contain less carbon dioxide at night than in the daytime, but by themselves are too few to prove this. However, later and more extensive work with *Vicia* makes the point clear.

To determine definitely the effect of darkness on carbon dioxide content of leaves, *Vicia faba* was used. Its paired leaflets made it especially suitable. One leaflet of a pair was analyzed at the time the plant was darkened, the other after the plant had been dark for the desired length of time. As a check on the method several pairs of leaflets were analyzed without having been subjected to any dark treatment, the second leaflet being analyzed as soon as possible after the first analysis. These data are given in table IV, experiment 1. The odds in favor of any significant difference between the

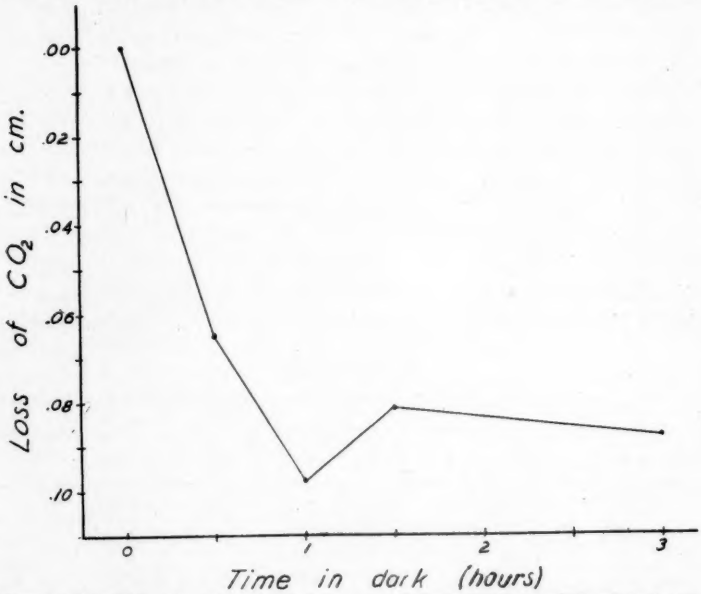

FIG. 3. Graph showing loss of CO_2 in leaves of *Vicia* during periods of darkness.

TABLE IV

PAIRED DATA FOR *Vicia* TO DEMONSTRATE EFFECTS OF DARKNESS ON CO_2 IN LEAVES

EXPERIMENT	IN LIGHT				TREATMENT	AFTER TREATMENT				
	NO. OF RUNS	AV. TOTAL VOLUME	AV. $CO_2 \pm \sigma_M$	CO_2*		AV. TOTAL VOLUME	AV. $CO_2 \pm \sigma_M$	CO_2*	D_{CO_2}	D/σ†
				%				%		
1	30	21.03	0.322 ± .03	1.53	None	21.44	0.323 ± .03	1.51	.001	− 0.03
2	40	19.53	0.357 ± .03	1.83	½ hr. dark	19.85	0.293 ± .02	1.47	.065	2.1
3	49	22.28	0.339 ± .02	1.52	1 hr. dark	22.93	0.241 ± .02	1.05	.098	3.4
4	30	18.52	0.346 ± .02	1.87	1½ hr. dark	20.56	0.264 ± .02	1.28	.082	2.9
5	30	16.57	0.298 ± .02	1.79	3 hrs. dark	17.91	0.210 ± .01	1.17	.088	4.3
6	29	18.56	0.398 ± .03	2.14	1 hr. dark (2°–5° C. above greenhouse temperature)	18.76	0.338 ± .02	1.80	.060	1.4

* The percentage values must not be construed as giving the percentages of CO_2 in the gases actually emerging from the leaves. Such percentages are undoubtedly higher than those given here. See text pages 147–148.

† When D/σ is 2.1 the odds in favor of the significance of D are 27 : 1.

means of the two series of data are far below 2 : 1, as calculated by D/σ. Any significant difference between the averages of the carbon dioxide contents of two series of paired leaflets of *Vicia faba* must, therefore, be due to the treatment given the plants between the times of picking of the two leaflets of each pair.

The plants of *Vicia* were darkened by being put in closed cupboards. The lengths of treatment were ½, 1, 1½, and 3 hours. The data obtained from these various groups of experiments are given in table IV (experiments 2–5). The results of the various groups are summarized graphically in figure 3.

The curve as drawn in this graph is of necessity only relative. It can be made nearly quantitative, but only if both light and temperature are controlled throughout the experiments employed in deducing the curve. (This is true, indeed, of all work reported in this paper.) From the present work it is clear that light influences the amount of carbon dioxide. There have been strong indications as time passed that temperature, too, affected the amount. Though the precise points on the curve cannot be considered fixed, it is reasonably certain that the general form of the curve will stand. To be sure the differences between adjacent points obtained by work on treated leaves are not significant statistically. However, it seems logical that the ½-hour point should be intermediate between the 0 and 1-hour points. The attainment of equilibrium, as denoted by the slight difference between the 1½- and 3-hour points, is also to be expected. That this equilibrium value should be below the zero point is supported by the data in table III, for the run of February 27–28. Here for three kinds of plants the night carbon dioxide average is less than that for the day.

In the drawing of the graph the zero point was arbitrarily assumed to be constant, and the other points were plotted as differences. That is, each check was assumed in its turn to be zero, and the difference between that check and the corresponding treated leaves was plotted directly on the graph. Such a method was necessitated by the existence of a certain amount of variation between the carbon dioxide values of the various checks.

The data obtained make it very certain that, under the conditions involved in these experiments, less carbon dioxide can be extracted from *Vicia* leaflets by a vacuum after those leaflets have been in the dark half an hour or more than can be extracted from similar leaflets in the light. In every case studied, the difference between the carbon dioxide contents of lighted and darkened *Vicia* leaflets was statistically significant. That such a decrease in extractable carbon dioxide occurs in other plants is indicated by the data from the 24-hour run involving *Tropaeolum, Vicia,* and *Beta;* by the work on the white leaves of *Salix* and *Rosa;* and by the work on white and variegated leaves of *Pelargonium.*

In all of the *Vicia* experiments just mentioned the darkened plants were

without doubt several degrees cooler than the lighted ones. This made it impossible to say whether the effect was due to change in light or in temperature conditions. An attempt was made to separate these two by putting the plants for 1 hour into a dark chamber which was 2°–5° C. warmer than the greenhouse. The temperature of lighted leaves (not in direct sunlight), as measured by a thermocouple, was not more than 1°–2° C. above the greenhouse temperature. Therefore, the darkened leaves were in this case probably warmer than the lighted ones. Nevertheless, there was a decrease in extractable carbon dioxide when the plants were darkened (experiment 6, table IV). Unfortunately, this decrease is not statistically significant. Neither is it as large as the decrease in the earlier lot of 1-hour darkened leaves. Its existence suggests very strongly, however, that part of the decrease in carbon dioxide in the earlier experiments, if indeed not all of it, was due to a change in light conditions.

Reference has been made to experiments involving white leaves. Three kinds of plants, *Salix, Rosa,* and *Pelargonium,* were used in these experiments. The *Salix* leaves were white from lack of some nutrient element, probably iron. The *Rosa* and *Pelargonium* leaves were genetically white. Too few white leaves were available to provide indisputable results. The indications are very strong, however, that the carbon dioxide content of white leaves decreases in the dark just as does that of green leaves. The data from these white-leaf experiments are summarized in table V. The data found there for green leaves were collected as checks on the white-leaf data, since no work had been done previously with any of the three species concerned.

The supplies of *Salix* and *Rosa* leaves were entirely too small to furnish statistically satisfactory results. It is interesting, however, that the white leaves of each showed a greater decrease of extractable carbon dioxide in the dark than did the corresponding green leaves.

The work with *Pelargonium* indicates strongly that the carbon dioxide contents of white and of green (in this case, variegated, but mostly green) leaves are the same and are affected similarly by light and heat. This is rather evident from table V, but is more striking when it is realized that in the white-leaf average for carbon dioxide in darkened leaves, is included one abnormally high reading of 1.07 cm. If this one reading is omitted, D_{CO_2} becomes 0.071, which is nearly the same as the value for mostly-green leaves; D/σ becomes 1.97, which gives odds of 19:1 in favor of the significance of D_{CO_2}. Lack of more white leaves of *Pelargonium* prevented further work of this sort.

As can be seen from table V, nearly twice as much gas was obtained from the white leaves of *Pelargonium* as from the green ones, yet the amounts of carbon dioxide were about the same. The larger total volumes of the white leaves were undoubtedly correlated with the leaf structure. The white leaves

TABLE V

EFFECT OF CHLOROPHYLL ON CO_2 ABSORPTION BY LEAVES

KIND OF PLANT	IN LIGHT				AFTER 1 HOUR IN DARK			
	NO. OF RUNS	AV. TOTAL VOLUME	AV. $CO_2 \pm \sigma_M$	CO_2*	AV. TOTAL VOLUME	AV. $CO_2 \pm \sigma_M$	CO_2*	D_{CO_2}
				%			%	
Salix								
White	6	10.01	0.122 ± .02	1.2	10.66	0.093 ± .03	0.9	0.029
Green	6	10.21	0.190 ± .05	1.9	9.03	0.198 ± .03	2.2	−0.008
Rosa†								
White	9	12.11	0.450 ± .06	3.7	13.68	0.354 ± .05	2.6	0.096
Green	6	16.59	0.508 ± .06	3.1	15.49	0.503 ± .07	3.3	0.005
Pelargonium‡								
White	26	19.88	0.347 ± .02	1.7	20.32	0.307 ± .04	0.9	0.040
Green	26	11.90	0.364 ± .02	3.1	13.12	0.297 ± .03	2.3	0.067

* Percentage values must not be construed as giving the percentage of CO_2 in the gases actually emerging from the leaves. Such percentages are undoubtedly higher than those given here. See text pages 147–148.

† The dark-treated roses, white and green, were analyzed an hour or more after nightfall had darkened them naturally. Such analyses were made on the same day as, but 6–12 hours after, the analyses of rose leaves in the light.

‡ For white *Pelargonium* $D/\sigma = 0.86$; for green $D/\sigma = 1.97$.

were somewhat larger, more hairy, and distinctly more succulent than the green ones. The white ones seem to have held more air on their surfaces and in their intercellular spaces because of these structural modifications. The modifications seem not, however, to have affected the carbon dioxide-holding power of the leaves.

Results and discussion

From the findings of this research three facts stand out. First, brightly lighted leaves contain a considerable amount of carbon dioxide which can be extracted and measured by the methods herein described; second, the amount of extractable carbon dioxide in leaves is no higher at night than in the daytime; and third, leaves lose carbon dioxide when taken from bright light and placed at once in darkness.

There has been developed a tentative theory to explain the presence of much carbon dioxide in lighted leaves, and the disappearance of some of that gas in the dark. The effect of temperature is so little known that no effort has been made to include an explanation of its action. It is obvious, however, that light is an important factor.

It seems impossible that all of the carbon dioxide found, at least 2 per cent. in some cases (table V), should exist as gas in the intercellular spaces. If the intercellular air contained 2 per cent. carbon dioxide, it would be impossible for leaves to obtain any of that gas from air, because the diffusion gradient would be in the wrong direction.

Since the carbon dioxide cannot be free, it must be bound in some way. Since a great deal of it can be rapidly extracted by a vacuum of approximately 0.01 atmosphere, it must be loosely bound. It might conceivably be bound in any of three ways: by solution, by adsorption, by loose chemical combination. It is very unlikely that the gas is in solution. A leaf weighing 0.5 gm. (and the *Vicia* leaflets used weighed only 0.2–0.3 gm.) would dissolve from air in its contained water only about 0.00015 cc. of carbon dioxide at room temperature, whereas the amount found experimentally was 50 times that much. Moreover, the decrease of carbon dioxide in the dark cannot readily be explained on the basis of solution; light should not have any pronounced effect on the solubility of a gas, and the drop in temperature occurring in the dark should increase solubility, not decrease it. It seems impossible on the basis of present data to determine which of the remaining two explanations is the better, whether the gas is adsorbed or loosely combined.

It seems probable from the present work that the material holding the carbon dioxide is either activated by light to allow it to adsorb the gas, or it is produced in the presence of light, undergoing subsequent combination with the gas. Temperature may conceivably be a supplementary agent influencing the production or activation of such a substance. In the absence of light

the material either holds the gas so firmly that a vacuum will not extract it, or more probably does not hold it at all, so that the gas is rapidly lost by the leaf.

The curve in figure 3 suggests that there are at least two factors affecting the amount of carbon dioxide in leaves. When a leaf is darkened, the light-activated carbon dioxide-holding mechanism releases its carbon dioxide. This occurs rapidly but not all at once. The resulting drop in extractable carbon dioxide is shown by the first and descending arm of the curve in figure 3. Respiration is constantly producing carbon dioxide, however, and after a time the carbon dioxide content of the leaf reaches a sort of equilibrium value, at which time the production of that gas is just balanced by its loss. The assumption is that then the dark-acting carbon dioxide-holding mechanism of SPOEHR and MCGEE (**8**) and of others is saturated. (SPOEHR and MCGEE used dried leaf material, and found that when moistened it would absorb much carbon dioxide in the dark.) It is difficult to see why the amount of carbon dioxide should become temporarily less than that in equilibrium with respiration. Perhaps the apparent minimum is due only to experimental error. If, however, the carbon dioxide is held more tightly in the dark, rather than being freed, the minimum may be a true one. If so, the subsequent rise represents the accumulation of carbon dioxide from respiration, until finally the equilibrium value is reached.

The most obvious assumption in connection with the light-activated absorption of carbon dioxide is that it depends in some way on chlorophyll. It was to test such an assumption that the white-leaf experiments were performed. As stated on page 151, the carbon dioxide contents of white leaves seem subject to the same variations under the same conditions as are the contents of green leaves. Hence, chlorophyll is not a factor concerned in the light-activated absorption of carbon dioxide by leaves.

This increased carbon dioxide content may be connected in some way with photosynthesis. On the other hand, it is conceivable that the carbon dioxide comes from an intermediate unstable product of respiration, whose formation is induced by light.

Few papers dealing with the extracted gases of leaves have been found. That by BOUSSINGAULT (**1**) does not give data in any such form that they can be compared with those presented here. The papers by GRÉHANT and PEYROU (**3, 4**) and by PEYROU (**7**) are pertinent, but difficult of interpretation. Their work seems to be the only extensive study devoted to the gases actually inside of leaves. Some of their data support the work here reported; other data contradict it. Probably their data cannot be considered very seriously either in support or in contradiction of this work.

Two factors make it difficult to compare satisfactorily their earlier experiments with these recent ones. For one thing, there seems considerable doubt

as to the accuracy of the work of GRÉHANT and PEYROU. Their extraction was slow, and involved the heating of the leaves. These factors together probably caused respiration to influence markedly the composition of the gases obtained, concealing completely the original O_2/CO_2 ratio. Carbon dioxide frequently formed 50 per cent. of the gases.

Assuming that the method was exact, however, one meets great difficulty in interpreting the data. For the most part, the experiments were not paired, they were not run in rapid succession, and they did not even use leaves from the same plant. Moreover, when obtained, the data were calculated in such a way as to emphasize the oxygen variations, and not those of carbon dioxide.

Another paper with possible significance in regard to the work just reported was written by KOSTYTSCHEW (5) in 1921. He reports that leaves put into chambers with high carbon dioxide concentration (6–10 per cent.) show at first a high CO_2–O_2 ratio. Later, if photosynthesis is rapid, this drops to approximately 1. He suggests "dass eine bedeutende CO_2-Menge ohne entsprechende Sauerstoffausscheidung chemisch gebunden war." Later he says, "Höchtswahrscheinlich steht die hervorgehobene Tatsache im Zusammenhange mit der wichtigen Beobachtung von WILLSTÄTTER und STOLL hinsichtlich der CO_2-Bindung durch kolloidale Chlorophyllösungen."

In light of the work reported in the present paper, it seems very likely that KOSTYTSCHEW was dealing with the light-activated absorption of carbon dioxide. This is suggested by (1) the preliminary large absorption of carbon dioxide, (2) the lack of such absorption in darkened leaves (only two cases given), and (3) the release of most of this carbon dioxide only if the partial pressure of the gas became rather low. Although they support and supplement the work of the writer, the data presented by KOSTYTSCHEW cannot be considered definitely to prove the correctness of the present work.

Summary

A method has been developed whereby the gases in a single leaf can be extracted and analyzed for carbon dioxide. By the use of this certain data have been obtained, and from these data the following conclusions have been drawn.

1. Leaves in light or in dark contain much carbon dioxide.
2. Leaves in the light contain more carbon dioxide than those in the dark.
3. Carbon dioxide gas is not entirely or mainly in the intercellular spaces.
4. This gas cannot be held simply in solution.
5. Part of the carbon dioxide in lighted leaves is held by some light-activated mechanism which involves adsorption or loose chemical combination.
6. Chlorophyll is not concerned with this light-activated mechanism.

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EFFECT OF HYDROGEN-ION CONCENTRATION ON *CHLORELLA* PHOTOSYNTHESIS

ROBERT EMERSON AND LOWELL GREEN
(WITH TWO FIGURES)

Introduction

Aquatic green plants can assimilate carbon dioxide in solutions of widely varying hydrogen-ion concentration. Our purpose in investigating the rate of photosynthesis as a function of this factor was to clarify the interpretation of experiments in carbonate mixtures, where the concentrations of carbonate and bicarbonate ions and of free carbon dioxide all vary with the hydrogen-ion concentration. The relationships are shown in figure 1, where the percentage of total carbon dioxide in each of these three forms is plotted against the pH. The range covered by the carbonate mixtures is to the right of pH 8. From here the proportion of bicarbonate, which is at first maximal, declines with increasing pH, and the carbonate, extremely low at first, rises gradually to its maximum somewhere beyond pH 12. At the same time the proportion of free carbon dioxide declines

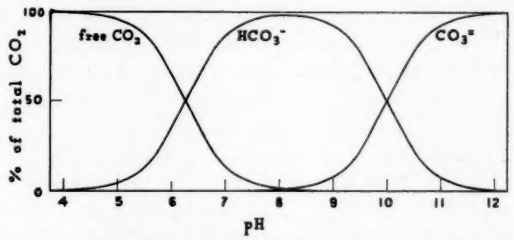

FIG. 1. Proportion of total carbon dioxide in each of the three forms, free carbonic acid ($CO_2 + H_2CO_3$), bicarbonate ions, and carbonate ions as a function of hydrogen-ion concentration.

from its already very low value at pH 8, and becomes insignificant at pH 9. Nevertheless, if the carbonate and bicarbonate concentrations are high enough, the concentration of free carbon dioxide remains appreciable, for purposes of measuring photosynthesis, down to pH 11. If the mixtures are prepared by mixing M/10 solutions of carbonate and bicarbonate, then at pH 9 the concentration of free carbon dioxide will be sufficient to "saturate" photosynthesis, that is to say, cells in this concentration of carbon dioxide will show the maximum rate of photosynthesis of which they are capable. This rate will be independent of even relatively large changes of concentration. At pH 10, obtained according to figure 1 by mixing equal parts of carbonate and bicarbonate, the concentration of free carbon

dioxide will be "limiting" for photosynthesis, or so low that the rate of photosynthesis is now a function of the concentration.

The solutions made by mixing M/10 carbonate and bicarbonate have a fair buffering capacity for carbon dioxide, a considerable amount of which may be used by assimilating cells without much change in concentration. WARBURG (**17**) prepared a series of carbonate-bicarbonate mixtures covering the range of carbon dioxide concentrations physiologically important for green plants, and used these mixtures to study photosynthesis as a function of the concentration of free carbon dioxide, on the assumption that among the several variables in the mixtures this factor alone was significant for photosynthesis. Although this assumption has been questioned by plant physiologists from time to time, there has been a tendency to accept NATHANSOHN'S (**10**) evidence in support of it (*cf.* also BENECKE and JOST, **4**, p. 201). JAMES (**9**) has discussed the possible influence of various factors in the carbonate mixtures, but his experimental results appear to support NATHANSOHN'S opinion, that the determining factor for assimilation is the concentration of free carbon dioxide. The work of OSTERHOUT and DORCAS (**11**) and OSTERHOUT and HAAS (**12**) may also be regarded as giving support to this viewpoint. However, WARBURG'S curves for *Chlorella* photosynthesis as a function of carbon dioxide concentration, and certain of the phenomena he observed in connection with temperature coefficients and the action of inhibitors, are in disagreement with results obtained by VAN DEN HONERT (**7**) and VAN DER PAAUW (**13, 15**) with *Hormidium*. In the *Hormidium* experiments the cells did not come in direct contact with carbonate mixtures, but were kept moistened with culture fluid. Both VAN DEN HONERT and VAN DER PAAUW have made several objections to WARBURG'S technique, particularly to the alkalinity of the carbonate mixtures, but VAN DER PAAUW (**14**), after comparing his own method with WARBURG'S, concluded that some of the disagreements in results were due to real physiological differences between *Hormidium* and *Chlorella*, and that the methods were of about equal value. Unfortunately his method was applicable only to the range of saturating carbon dioxide concentrations. The most striking differences between WARBURG'S results and VAN DEN HONERT'S were in the range of limiting concentrations of carbon dioxide, where the characteristics of photosynthesis are of particular importance because they should reveal something of the reactions entered into by carbon dioxide. But when the concentration of free carbon dioxide is varied by using different carbonate mixtures, after the method of WARBURG, the concentrations of carbonate and bicarbonate as well as hydrogen ions are subject to the variations shown in figure 1, and it is by no means certain that these three factors are without influence on photosynthesis. Recent work of ARENS (**1, 2, 3**) shows that some water plants may absorb both carbonate and bicarbonate ions, and he makes it appear likely that carbon dioxide absorbed in these forms can be used for assimilation.

The concentration of free carbon dioxide in the carbonate mixtures can be varied by making the various mixtures more or less concentrated, as well as by mixing different proportions of a given concentration of carbonate and bicarbonate. For a particular mixture, the pH does not vary much with the concentration, while the free carbon dioxide is roughly proportional to it. Some independent control of the several factors is possible in this way, but the available range is small because the more dilute mixtures have insufficient buffering capacity to permit accurate measurements of assimilation, and the more concentrated mixtures are unphysiological. Measurements conducted in two series of mixtures of M/10 and M/20 concentration show that for a given calculated concentration of free carbon dioxide, the rate of photosynthesis is always higher in the more dilute mixture. This suggests that other factors besides the concentration of free carbon dioxide determine the rate of photosynthesis in the carbonate mixtures. Values published by various authors for the constants required to calculate the concentration of free carbon dioxide do not agree well, and it is possible that our calculated concentrations are not quite correct, but they are probably correct relative to one another, so it is hardly possible that errors in the constants used are responsible for our results. Differences in the ionic strength or osmotic pressure of the mixtures are also unlikely to be directly responsible because *Chlorella* photosynthesis is indifferent to these factors over a wide range. The other variables are the concentrations of carbonate, bicarbonate, and hydrogen ions. Since the concentration of the latter establishes the relative concentrations of carbonate and bicarbonate ions as well as of free carbon dioxide, the importance of studying the influence of pH on photosynthesis is evident.

Although conditions in the carbonate mixtures are of special interest, they do not offer a good starting point for this investigation because of the close interdependence of all the variables under consideration. Greater freedom of control of variables is achieved by working on the acid side of pH 8, in phosphate instead of carbonate buffers. It becomes possible to establish the concentration of free carbon dioxide by saturation with a fixed partial pressure of carbon dioxide, and the bicarbonate concentration alone will vary with the pH. The carbonate ions become negligible on the acid side of pH 8. It is safe to assume that the concentrations of the two phosphate ions, by means of which the pH is controlled, are without effect on the rate of photosynthesis over the range in our experiments.

By going from carbonate to phosphate buffers we have achieved a certain simplification, but we have also added an important limitation, and turned away from our original problem. The limitation concerns the available range of carbon dioxide partial pressures at which we can study photosynthesis by the usual manometric technique. The most important feature of the carbonate mixtures is that they maintain low partial pressures of carbon

dioxide nearly constant even while considerable amounts are used for photosynthesis. It is thus possible to follow the rate of photosynthesis for long periods at carbon dioxide partial pressures far below that of ordinary air. But if acid phosphate at pH 4.6 is saturated with ordinary air, it will not contain enough carbon dioxide to make a single rate measurement. A quantity of cells sufficient to give satisfactory manometer readings will consume all the carbon dioxide in the first few moments of illumination. Therefore in experiments using phosphate buffers, we are limited to concentrations of carbon dioxide so large that the amount withdrawn by the assimilating cells makes no significant change in the total concentration. The lowest practicable carbon dioxide concentration is 0.5 per cent. The upper limit is about 5 per cent., because higher concentrations begin to have a narcotic effect on assimilation. But from 5 to 0.5 per cent. the rate of photosynthesis is independent of carbon dioxide partial pressure, while our original interest was in the range of limiting carbon dioxide concentrations. Several years' efforts have convinced us that new methods are required to investigate the influence of hydrogen-ion concentration over this range, and our attempts along these lines have not been entirely successful. We believe, however, that the simpler conditions prevailing in the phosphate buffers are of sufficient interest to justify presentation of our results, and that an understanding of the behavior of photosynthesis under these conditions should precede the investigation of the more complex relationships beyond pH 8.

Investigation

Photosynthesis was measured manometrically in the usual way, with *Chlorella vulgaris* or *C. pyrenoidosa* cells suspended in M/25 potassium phos-

TABLE I

RETENTIONS $\left(\frac{\Delta u}{\Delta p}\right)$ AND pH VALUES OF PHOSPHATE BUFFERS, AT 20° C.

Composition	Parts $\frac{M}{25}$ KH_2PO_4	10	9	5	1
	Parts $\frac{M}{25}$ K_2HPO_4	0	1	5	9
Before saturation	pH calculated	....	6.0	6.9	7.8
	pH colorimetric	4.6	6.0	6.9	7.7
Saturated with 0.5% CO_2 in air	pH, colorimetric	4.6	6.0	6.8	7.6
	$\frac{\Delta u}{\Delta p}$, calculated	0	0.05	0.28	0.86
Saturated with 5% CO_2 in air	pH, colorimetric	4.6	5.9	6.6	7.0
	$\frac{\Delta u}{\Delta p}$ calculated	0	0.03	0.13	0.21
	$\frac{\Delta u}{\Delta p}$ by dilution	....	0.025	0.14	0.23
	$\frac{\Delta u}{\Delta p}$ by slight acidification	....	....	0.12	0.22
	$\frac{\Delta u}{\Delta p}$ by excess acidification	....	0.026	0.12	....

phate buffers. The compositions of the four mixtures used are shown in the two top rows of table I. The next two rows show the pH of each mixture before saturation with carbon dioxide in air, both calculated and determined colorimetrically with indicators. The fifth and seventh horizontal rows show the pH (determined colorimetrically) when the mixtures were saturated with 0.5 and 5 per cent. carbon dioxide in air.

The rate of photosynthesis was computed from the pressure change on the manometers, which were read at 5-minute intervals. In the potassium dihydrogen phosphate solution at pH 4.6, the bicarbonate concentration is negligible (fig. 1) and the calculation of the gas exchange is made in the ordinary way (WARBURG **18,** p. 104, equation 6) from the gas and fluid volumes, the temperature, and the solubility of the exchanged gases in water. In the buffers containing dipotassium hydrogen phosphate, the dissociation of carbonic acid becomes appreciable, and the resulting bicarbonate ions alter the behavior of the solution. When carbon dioxide is used for photosynthesis, part is supplied by decomposition of the bicarbonate. The result is that a given fall in carbon dioxide pressure represents a larger consumption of carbon dioxide than if no bicarbonate were present, and another constant must be added to the expression for calculating gas exchange. WARBURG has called this the chemical "retention" for carbonic acid, and developed three methods for measuring retention in blood serum which are directly applicable to our phosphate buffers when these are saturated with 5 per cent. carbon dioxide in air (**18,** p. 208–212) The last three rows of table I show retentions, $\frac{\Delta u}{\Delta p}$ in WARBURG's notation, for our mixtures by the three different methods.

WARBURG has also shown (**18,** footnote p. 207) how the retention in phosphate can be calculated from the dissociation constants of carbonic and phosphoric acids. While direct measurement is to be preferred whenever possible, the calculated retentions are given for comparison (row 8, table I), directly above the experimental retentions. For each pH the retentions obtained in the different ways agree well with one another.

When the phosphate buffers are saturated with 0.5 instead of 5 per cent. carbon dioxide the bicarbonate concentrations become too low for satisfactory application of the methods for measuring retention, so for the lower partial pressure of carbon dioxide table I shows only calculated retentions (row 6). The agreement between the calculated and experimental retentions for the higher partial pressure is sufficient to give confidence in the method of calculation.

For calculating retentions, as well as for calculating the concentrations of bicarbonate ions in the phosphate solutions, pK'_1 and pK'_2 for carbonic acid were assigned values of 6.25 and 10 respectively, at 20° C. For calcu-

lating carbon dioxide concentrations in the carbonate mixtures, the value of pK'_1 was changed to 6.22, while the value of 10 for pK'_2 was retained. To select from the literature the best limiting values for the constants, and to apply the appropriate corrections for ionic strength, has entailed a number of choices which may seem arbitrary. While we would like to support our choices by a discussion of the literature, this is unnecessary for the purposes of the present paper and is reserved until a later date. For the present it is sufficient to explain that the value of pK'_1 was obtained by correcting SHEDLOVSKY'S and MACINNES' (**16**) figure of 6.39 for pK_1, for ionic strength according to an equation used by HASTINGS and SENDROY (**6**). Our value of pK'_2 was obtained directly from WEIDER'S (**21**) determinations.

Under the simple conditions of our experiments, it is safe to assume the photosynthetic quotient (γ in WARBURG'S equation 6, referred to above) of *Chlorella* is unity. It is therefore possible to check the retention figures by using WARBURG'S differential method (**18**, pp. 104–108) for the photosynthesis measurements. Each determination is made in duplicate, in two vessels of approximately equal volume, containing equal quantities of cells, but unequal volumes of fluid. If the retentions are correct, the computed gas exchange in each pair should agree.

Table II shows the rates of photosynthesis of *C. pyrenoidosa* in 5 per cent. carbon dioxide from pH 4.6 to 7. The concentrations of hydrogen and bicarbonate ions are shown in the second and third horizontal rows. The rates of photosynthesis for two different experiments (a and b) are shown

TABLE II

RATES OF PHOTOSYNTHESIS, IN CUBIC MILLIMETERS OF OXYGEN PER HOUR PER CUBIC MILLIMETER OF CELLS, CORRECTED FOR RESPIRATION, AT 20° C., FOR *Chlorella pyrenoidosa* CELLS SUSPENDED IN VARIOUS PHOSPHATE MIXTURES SATURATED WITH 5 PER CENT. CARBON DIOXIDE IN AIR. CONCENTRATION OF DISSOLVED FREE CARBON DIOXIDE IS 2 MILLIMOLES PER LITER. ROW (a) GIVES RATES FOR AN OLD CULTURE; ROW (b) FOR A FRESH CULTURE

pH	4.6	5.9		6.6		7.0	
$[H^+]$, moles per liter $\times 10^7$	250	12.6		2.5		1.0	
$[HCO_3^-]$, moles per liter $\times 10^5$	5	100		500		1200	
Volume of suspension, ml.	7	7	3	7	3	7	3
Rates of photosynthesis (a)	12.4	12.4	11.6	12.7	12.8	12.4	13.1
Rates of photosynthesis (b)	28.6	27.4	27.5	28.7	29.0	28.6	29.7

in the two bottom rows. Except for the determinations at pH 4.6, all rates were measured in duplicate with two different fluid volumes (row 4, table II). The good agreement between the duplicate determinations shows that the retention figures are fairly accurate. In spite of the wide range of con-

centrations of bicarbonate and hydrogen ions covered in this experiment, the rate of photosynthesis remains remarkably constant. The small variations are random and of no significance.

To cover a somewhat wider range of pH, a carbonate buffer of pH 8.9 was added to the phosphate series. The computed concentration of free carbon dioxide in this mixture corresponds to a partial pressure of about 0.5 per cent. carbon dioxide in air, so to maintain the concentration comparable throughout the series, the phosphate buffers were saturated with this gas mixture. The exact composition of the gas mixture is of no importance because although in the neighborhood of pH 8.5 the rate of photosynthesis varies a little with the composition of the carbonate mixture, we shall show that in phosphate it is constant over the range of carbon dioxide partial pressures covered in these experiments (see fig. 2 and accompanying discussion).

Table III shows rates of photosynthesis for two experiments covering the range of pH 4.6 to 8.9, one for *C. pyrenoidosa*, the other for *C. vulgaris*. It will be recalled that for 0.5 per cent. carbon dioxide the retentions were computed, and not measured experimentally as for table II. The agreement between duplicate determinations nevertheless remains satisfactory. Although the rates of photosynthesis are a little scattered, they show no tendency to vary consistently with the concentration of hydrogen, bicarbonate, or carbonate ions. There is therefore no indication that any one of these factors plays a part in determining the rate of photosynthesis, at least down to 0.5 per cent. carbon dioxide.

During the measurements of photosynthesis in phosphate saturated with 0.5 per cent. carbon dioxide, the latter was used up so rapidly that its concentration must have undergone considerable change even in the course of the brief rate determinations (10 or 15 minutes). It was stated above that in these experiments the exact concentration was of no importance. This was established by using a technique developed by WARBURG and KUBOWITZ (**19**) for the study of respiration at low partial pressures of oxygen. They admitted a small amount of oxygen to a vessel containing a suspension of respiring cells, and followed the disappearance of oxygen on a differential manometer from minute to minute until it was gone. They could then compute the concentration of the oxygen remaining at any given time, and the corresponding rate of respiration. Adapting the technique to photosynthesis, we passed known amounts of carbon dioxide into a vessel containing a suspension of *Chlorella* cells. A saturating light intensity was used for these experiments, so that, at the higher carbon dioxide concentrations, the only external factor limiting photosynthesis was temperature. The rates of photosynthesis were calculated for various carbon dioxide concentrations in the same way that WARBURG and KUBOWITZ (**19**) calculated respiration. For

TABLE III

RATES OF PHOTOSYNTHESIS, IN CUBIC MILLIMETERS OF OXYGEN PER HOUR PER CUBIC MILLIMETER OF CELLS, CORRECTED FOR RESPIRATION, AT 20° C., FOR TWO SPECIES OF *CHLORELLA*, SUSPENDED IN VARIOUS PHOSPHATE MIXTURES SATURATED WITH 0.5 PER CENT. CARBON DIOXIDE IN AIR, AND IN A CARBONATE MIXTURE OF pH 8.9 IN EQUILIBRIUM WITH THE SAME CARBON DIOXIDE PARTIAL PRESSURE. CONCENTRATION OF DISSOLVED FREE CARBON DIOXIDE IS 0.2 MILLIMOLES PER LITER

pH		4.6	6.0		6.8		7.6		8.9
$[H^+]$, *moles per liter* $\times 10^9$		25,000	1000		158		25		1.3
$[HCO_3^-]$, *moles per liter* $\times 10^6$		5	120		800		5000		92,000
Volume of suspension, *ml.*		7	7	3	7	3	7	3	7
Rates of photosynthesis	*C. vulgaris*	12.2	10.9	11.7	13.4	14.9	13.1	14.0	12.1
	C. pyrenoidosa	11.4	11.6	12.5	12.4	13.5	11.9	12.7	10.8

C. pyrenoidosa cells suspended in M/25 phosphate at pH 4.6, the rate of photosynthesis is plotted against carbon dioxide concentration in figure 2. Its perfect constancy over almost the entire range of carbon dioxide concentrations is remarkable, especially when contrasted with the corresponding plot of measurements made in carbonate mixtures (*cf.* WARBURG, **17**, p. 254, fig. 10; EMERSON and ARNOLD, **5**, p. 409, fig. 10). In these cases the upper part of the

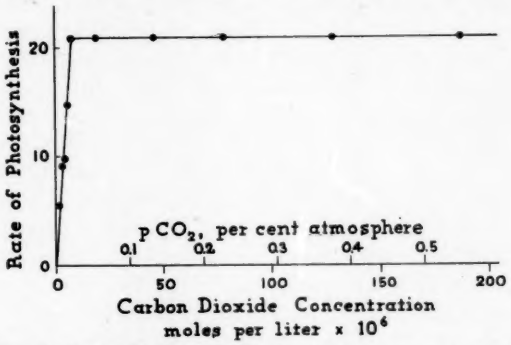

FIG. 2. Rate of photosynthesis in M/25 phosphate at 25° C. in cubic millimeters of oxygen per hour per cubic millimeter of cells, as a function of carbon dioxide concentration in the suspending fluid in moles per liter $\times 10^6$. Auxiliary scale on upper side of axis of abscissae shows the partial pressures of carbon dioxide in equilibrium with the corresponding concentrations. The differential manometer method described in the text was used to obtain this curve.

curve is never quite horizontal. Figure 2, although made with the same species of *Chlorella* as WARBURG'S, more closely resembles VAN DEN HONERT'S curve for *Hormidium* (**8**, p. 1017, fig. 3). Our curve shows photosynthesis remaining constant even down to a slightly lower partial pressure than in his experiment. There can be no doubt that in this particular case the difference between WARBURG'S results and VAN DEN HONERT'S is due not to physiological differences between the organisms used, but to differences in the methods. VAN DEN HONERT'S cells never came in contact with carbonate mixtures, and the conditions for his experiment were comparable with those for figure 2. The difference between WARBURG'S carbon dioxide curve and our figure 2 supports the conclusion drawn earlier in this paper, from the experiments in carbonate mixtures at different concentrations, that other factors besides the concentration of free carbon dioxide determine the rate of photosynthesis in the carbonate mixtures. These mixtures do not give us a true picture of the influence of carbon dioxide concentration on photosynthesis.

The curve in figure 2 is taken from one of many attempts to measure photosynthesis in phosphate at limiting concentrations of carbon dioxide. While there is no doubt concerning the horizontal portion, which continues

straight to 5 per cent. carbon dioxide, ten times the range of concentration shown in the figure, the shape of the descending limb is uncertain because of limitations inherent in the method. It would probably be satisfactory if photosynthesis could be studied from the exchange of carbon dioxide alone, just as WARBURG and KUBOWITZ studied respiration from the exchange of oxygen alone. They achieved this by using alkali to absorb the carbon dioxide produced. In our photosynthesis experiments we have tried to use yellow phosphorus to absorb the oxygen produced, in order to measure photosynthesis from changes in carbon dioxide pressure. We were able to confirm the general belief that anaerobiosis in the dark does not interfere with the immediate start of photosynthesis when the light is turned on, but found that continued illumination in the presence of yellow phosphorus produced a slowly declining rate of photosynthesis. It is possible that the presence of phosphorus rather than the absence of oxygen was responsible for the injury, although one would be inclined to doubt this because the exposure of cells to the presence of yellow phosphorus in the dark for many hours is quite without effect on subsequent photosynthesis. In any case we were unable to obtain constant rates of photosynthesis at constant carbon dioxide concentrations if yellow phosphorus was present, and were therefore obliged to work under aerobic conditions. For experiments under aerobic conditions it is necessary to calculate the gas exchange from the combined change in pressure of both oxygen and carbon dioxide, assuming that they are exchanged in equal amounts. This is certainly a close approximation to the truth, and is entirely satisfactory for determinations made with ordinary open manometers. It is also satisfactory for the differential manometer experiments over the range covered by the horizontal portion of the curve in figure 2. But the vessel constant for the differential manometer is more sensitive to changes in the ratio of exchange of oxygen and carbon dioxide because for these experiments the fluid volume must be much smaller in proportion to the gas volume than for the ordinary manometer experiments, in order to keep the diffusion resistance as small as possible. This becomes important when the rate of photosynthesis begins to decline with declining carbon dioxide concentration because, although the photosynthetic quotient may remain perfectly constant until the last trace of carbon dioxide is gone, there are evidently other physiological processes which lead to the evolution of small amounts of oxygen in the light, and they may greatly alter the ratio of exchange of carbon dioxide and oxygen as the carbon dioxide concentration and photosynthesis approach zero. The actual amount of oxygen produced in these accessory processes is small, but sufficient to introduce considerable uncertainty into the points on the descending portion of the curve in figure 2. The chief disturbing process is nitrate reduction (*cf.* WARBURG and NEGELEIN, **20**), which may continue to produce oxygen long after the last traces of carbon dioxide are gone and photosynthesis has ceased. Sometimes this

happens even with cells previously starved for nitrate for many hours, and at other times the process seems to be wholly in abeyance. We are never certain that it has not disturbed the apparent course of photosynthesis somewhat, even when the pressure change appears to cease with the disappearance of the last traces of carbon dioxide. Our differential manometer experiments will tell us down to what partial pressure photosynthesis remains constant, but they fail to show exactly how the rate changes when it becomes limited by carbon dioxide concentration.

Summary and conclusions

For the present, then, our investigation of the influence of the hydrogen-ion concentration on photosynthesis stops at the upper range of carbon dioxide concentrations obtainable in carbonate mixtures, and leaves untouched the more interesting range of limiting concentrations. At saturating concentrations of carbon dioxide, neither the hydrogen- nor bicarbonate-ion concentration influences the rate of photosynthesis from pH 4.6 to 8.9. This pH range represents a change in concentration of nearly twenty thousand fold, in both cases. Nevertheless, there is good evidence that in the carbonate mixtures, where the carbon dioxide concentration is lower and the pH greater, other factors besides the concentration of free carbon dioxide play a part in controlling the rate of photosynthesis. In all probability these factors are the concentrations of bicarbonate and carbonate ions. This opinion is supported by our finding that the rate of photosynthesis in phosphate is remarkably constant from 5 per cent. atmospheric carbon dioxide down to less than 0.05 per cent., while over the same range of concentrations in carbonate mixtures, the rate is known to decline somewhat with declining carbon dioxide concentration.

We cannot say how great a change in internal pH is brought about by changing the external pH from 4.6 to 8.9. That there is some change in internal pH is indicated by the behavior of respiration, which seems to be about 30 per cent. higher at 4.6 than at 7 or 8.9. If we could establish the range of pH at the chloroplast over which photosynthesis is constant, it would help in forming opinions concerning the merits of proposed mechanisms of photosynthesis.

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SOIL MOISTURE AS AN INDICATION OF ROOT DISTRIBUTION IN DECIDUOUS ORCHARDS

F. J. Veihmeyer and A. H. Hendrickson
(WITH THREE FIGURES)

Introduction

The study of soil-moisture conditions in an orchard involves, among other things, two perplexing problems that the investigator should clearly understand before the routine work of securing the soil samples is started. One of these is: "What constitutes an adequate sample as regards depth?" And the other: "Where, in the plot, shall the samples be taken?" No two sets of conditions are identical, and the method of sampling used in one case may not give satisfactory results when used in another. It is possible, however, to secure satisfactory results in orchards of mature deciduous trees, provided the soil throughout the area sampled is reasonably uniform in texture, and is of such a nature that it is easily penetrated by roots. Some root stocks, such as the Japanese pear, do not seem to spread through the soil uniformly, and sometimes do not give satisfactory results even when planted on a homogeneous soil.

The experiments reported in this paper give the results of systematic samplings in 13-year-old peach, prune, and walnut orchards growing on a Yolo loam soil at Davis, California. The data show where, with respect to the trunk of the tree, the samples should be taken, and the depth of sampling necessary to secure an adequate record of the use of water by the tree. Inasmuch as the loss of moisture from the soil, below the depth that dries because of surface evaporation, is due, almost entirely, to use of water by the tree, the drying of the soil during the growing season may be used as an indication of the spread and depth of the root system. Data are also given from a 45-year-old pear orchard where the soil-moisture record plainly indicated the lack of uniform distribution of roots.

Plan of the experiment

During the 1935 season, soil samples were taken in one-foot increments to a depth of 6 feet at monthly intervals in one of the unirrigated plots of the experimental walnut orchard. Four sets of samples of 4 cores each were secured on each sampling date: the first at 8 feet from the trunk of the tree; the second at 12 feet; the third at 16 feet; and the fourth at 20 feet. The first samples were taken on April 24, and the last on October 29, 1935. As the trees were 48 feet apart, the sampling extended almost to the mid-point between the trees. At the time the samples were taken, there was a space of about 10 feet between the ends of branches of adjacent trees. The samples

were placed in tared sheet-metal cans fitted with tight covers, weighed, and then dried in the usual way at 110° C. for forty-eight hours.

In 1936 samples were taken at frequent intervals in another one of the unirrigated plots that entered the growing season with the soil wet to a depth of 12 feet. These samples were taken at three places in each plot, in one-foot increments to a depth of 9 feet, and in a 3-foot increment from 9 to 12 feet. They were taken between 8 and 12 feet from the trunk, the successive points of sampling being moved around the tree in a clockwise direction.

The work of the 1936 season also included an experiment similar to the one carried out in 1935, but was conducted on one of the irrigated plots. The samples were taken in one-foot increments to a depth of 6 feet, at 4, 8, 12, 16, 20, and 24-foot distances from the trunk of the tree. Only two sets of samples were obtained, one on June 12 while there was still readily available moisture remaining from the winter rains, and the second on October 20, while there was still available moisture from the second irrigation. This plot was irrigated on June 15, and again on August 6.

Similar experiments were carried on with peach and prune trees in irrigated plots. Inasmuch as peach and prune trees were planted 24 feet apart, the samples were taken in one-foot increments at 3-, 6-, 9-, and 12-foot spaces from the trunk, and consisted of 4 cores each, taken 90 degrees apart.

Experimental results

The results obtained in 1935 with walnuts are shown graphically in figure 1. The moisture contents of all samples obtained on April 24 at the beginning of the experiment were approximately at field capacity. The percentage of soil moisture in the top foot decreased uniformly during the first part of the growing season at all sampling distances from the tree. The curves for the individual samples flattened shortly before July 26, and showed a slight loss of moisture in the soil at this depth during August. The slight increase in moisture content late in the season was caused by rain which fell during October and which was held in the top foot.

The second foot likewise showed a fairly uniform decrease in soil moisture until about July 26, after which little, if any, extraction occurred, indicating that the permanent wilting percentage was reached. The results obtained in the third foot were essentially similar to those from the second. The individual samples, however, did not agree as closely as in the second foot, owing, probably to a slight variability in the texture of the soil found at this depth.

The moisture content of the fourth foot was also reduced fairly uniformly at the different sampling distances until July 26. Thereafter, the curves were close together and showed practically no extraction. In the

fifth and sixth feet the extraction of moisture seemed to be slightly more irregular than in the top four feet. At these depths the curves flattened out on August 27, after which extraction of moisture was very slow. It would seem that the readily available soil moisture was not exhausted in the fifth and sixth feet until about a month after it was in the top four feet.

It is interesting to note the comparatively small variation in the results whether the samples were taken 8, 12, 16, or 20 feet from the tree, which indi-

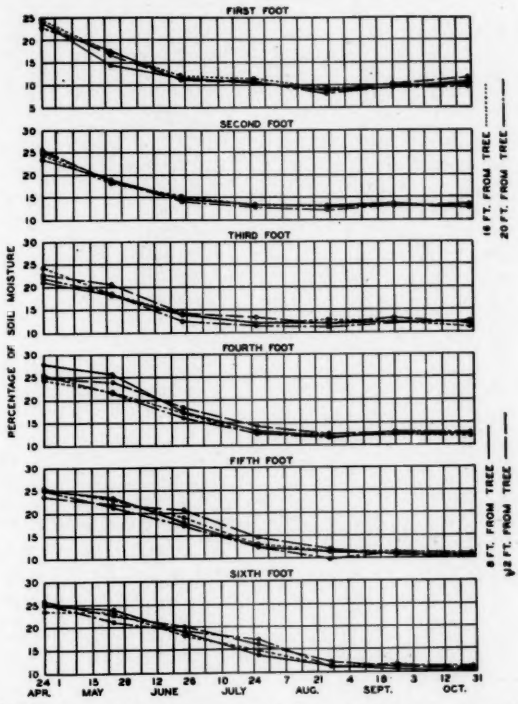

FIG. 1. Moisture conditions in upper six feet of soil in a walnut orchard, Davis, California, in 1935.

cates a fairly complete distribution of roots. The curves obtained from these experiments illustrate the fact that the readily available water at a given depth was extracted about as rapidly near the tree as it was farther away. In other words, the permanent wilting percentage at 20 feet from the trunk of the tree was reached as soon as it was at 8 feet. Furthermore, the permanent wilting percentage was reached in the top four feet about July 26, and about a month later in the fifth and sixth feet.

The results obtained by sampling a walnut orchard at specific distances

from the trunk of the tree in 1936 are given in table I. Each value is the mean of 4 samples taken 90 degrees apart. This plot, which was irrigated twice during the growing season, was sampled on June 12, and again on October 20. Close agreement was obtained at specific depths, regardless of the distance from the tree.

TABLE I

AVERAGE PERCENTAGE OF SOIL MOISTURE IN A WALNUT ORCHARD AT DIFFERENT DEPTHS AND DISTANCES FROM THE TREE, 1936

DISTANCE FROM TREE	DEPTH OF SAMPLE IN FEET					
	0 to 1	1 to 2	2 to 3	3 to 4	4 to 5	5 to 6
	Date of sample June 12					
4 feet	15.3	17.1	15.1	18.3	22.0	22.4
8 feet	14.8	16.4	14.6	18.6	21.4	22.9
12 feet	14.6	17.5	14.3	17.5	20.6	24.3
16 feet	13.7	16.3	15.6	17.6	20.7	23.5
20 feet	13.7	15.7	15.1	15.4	19.0	23.8
24 feet	14.0	15.7	15.5	15.6	17.7	23.1
Av.	14.3 ± 0.2	16.4 ± 0.2	15.0 ± 0.3	17.2 ± 0.3	20.2 ± 0.4	23.3 ± 0.5
	Date of sample October 20					
4 feet	12.9	14.8	13.2	14.8	14.8	14.1
8 feet	13.4	14.5	13.6	14.3	15.1	14.4
12 feet	12.8	14.9	13.9	13.7	15.0	14.9
16 feet	11.7	15.1	13.8	13.5	15.9	15.9
20 feet	11.8	14.1	14.2	13.9	14.8	15.2
24 feet	10.9	14.2	14.1	13.4	14.8	13.5
Av.	12.2 ± 0.2	14.6 ± 0.2	13.8 ± 0.2	14.0 ± 0.2	15.1 ± 0.3	14.7 ± 0.4

The samples in this plot were taken in each case before the permanent wilting percentage was reached. If the samples were not taken until several days or weeks after the permanent wilting percentage was reached, the results would probably be in agreement, inasmuch as the roots would have had ample time in which to use up the available supply; but in this case the agreement was close even though the samples were taken before the permanent wilting percentage was reached. Furthermore, the results obtained show the uniform use of water at varying distances from the trunk following the wetting of the soil by the even application of water in basins, as well as following the wetting of the soil by the winter rain. Where water is applied in furrows, the irregular distribution of moisture that often results, causes difficulty in interpreting the results secured by soil sampling.

The results obtained in 1936 by frequent sampling to a depth of twelve feet are shown in figure 2. The results obtained with this experiment agree essentially with those obtained in 1935 except that, inasmuch as the work was carried on in another unirrigated plot approximately 500 feet distant from the one used in 1935, the permanent wilting percentages are not the same in each plot. The extraction of moisture in the top foot was fairly

rapid in the early part of the season, but was slower during the latter part of May, and in June, because of light showers and an unusual number of cloudy days. Later in the season the moisture percentage (the sample contained the air-dried, surface soil) was reduced to about 7.5 per cent. The extraction of moisture from the second- and third-foot depths was similar to that from the first foot, the soil moisture being reduced to about the permanent wilting percentage July 15. The soil-moisture reduction in the fourth, fifth, and sixth feet was fairly rapid from the first sampling on April 17 to July 15, when the rate of extraction was materially decreased. After this date the extraction of water was slow. In other words the moisture at this depth was reduced to about the permanent wilting percentage on July 15.

In the seventh-, eighth-, and ninth-foot levels the extraction seemed to be

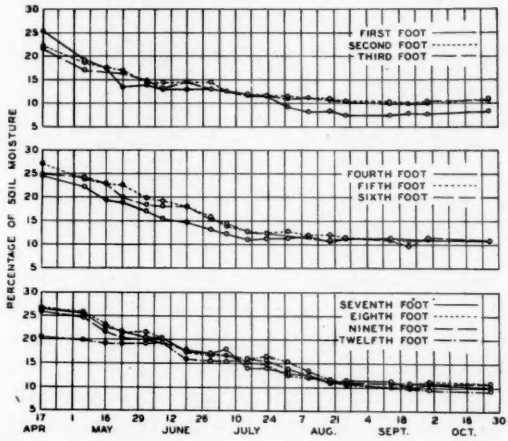

FIG. 2. Soil-moisture conditions in walnut orchard, Davis, California, in 1936.

slightly slower than it did in the top 6 feet of soil. Furthermore, the curves are somewhat more irregular because of variability in the soil. The downward trend of the curves, however, indicates the presence of a fairly dense root population. The permanent wilting percentage was reached August 19 which was about a month after it was reached in the top 6 feet.

The extraction curve for the soil between the 9- and 12-foot levels is somewhat irregular because of the frequency with which coarse textured material was encountered. Nevertheless, it shows that the soil moisture at this depth was reduced from 20.5 per cent. in the middle of April to about 10 per cent. by the middle of September. The reduced rate of extraction after September 14, even though temperatures were high, shows that the permanent wilting percentage was reached on that date.

The results obtained in the peach and prune orchards by sampling in one-foot increments 3, 6, 9 and 12 feet from the tree are given in table II.

TABLE II

AVERAGE PERCENTAGE OF SOIL MOISTURE AT DIFFERENT DEPTHS AND DISTANCES FROM THE TREE, JULY 18, 1936

DISTANCE FROM TREE	DEPTH OF SAMPLE IN FEET					
	0 to 1	1 to 2	2 to 3	3 to 4	4 to 5	5 to 6
	Peaches					
3 feet	10.0	11.5	10.8	11.2	11.7	12.6
6 feet	10.8	12.3	11.3	11.6	10.7	11.9
9 feet	10.0	11.6	11.2	11.3	11.5	11.6
12 feet	13.6	12.3	10.8	9.8	10.7	11.6
Av.	11.1 ± 0.1	11.9 ± .01	11.0 ± 0.1	11.0 ± 0.3	11.2 ± 0.2	11.9 ± 0.2
	Prunes					
3 feet	10.2	12.7	11.3	10.2	10.8	11.3
6 feet	11.0	12.2	10.6	11.1	11.0	10.9
9 feet	10.0	11.9	11.1	10.5	10.7	10.5
12 feet	9.2 –	11.8	11.4	10.6	10.6	10.2
Av.	10.1 ± 0.2	12.1 ± 0.1	11.1 ± 0.1	10.6 ± 0.2	10.8 ± 0.1	10.7 ± 0.1

The percentages given in the table are the averages of four samples taken from the north, east, south, and west sides of the tree at the distances and depths indicated. The agreement obtained in most cases was very close, as indicated by the small probable errors, and substantiate those from the walnut orchard. These results show what reasonably may be expected when soil-moisture studies are conducted with trees having adequate root distribution and growing on a fairly uniform soil.

On the other hand, while studying the water relations of pear trees in the central coast region of California, the results obtained by soil sampling in an unirrigated plot showed irregularities of considerable magnitude. The trees were on French pear root stocks and were about 45 years old at the time of the experiment. They were planted 20 feet apart on the square system. We assumed that, after 45 years, the soil under the trees was thoroughly permeated by roots at least to the depth ordinarily reached by this species. The soil, which was a Yolo loam, seemed to be uniform to a depth of 6 feet when the preliminary examination was made with the aid of a large post hole auger. As the season progressed, it was evident from the soil-moisture record obtained that either the roots were not uniformly distributed throughout the soil mass, or that layers of soil of different moisture holding properties occurred at varying depths. Careful examination of the samples secured with a soil tube showed considerable differences in texture at specified depths even when taken close to one another.

At the end of the 1934 season a trench, 6 feet deep, was dug about 4 feet

from one of the trees in the dry plot. It was then discovered that at a short distance below the cultivated layer there were distinct layers of soil differing rather widely in texture. In some of these layers there was an abundance of roots of different sizes, while in others, there was a scarcity.

A 12-inch square was laid off on the face of the newly dug trench approximately 2½ feet below the surface, directly in line with the trunk of the tree, and 36 samples, equidistant from each other, were taken within the 12-inch square by means of a short soil tube. The moisture record was obtained by drying and weighing in the usual manner. The results, expressed as percentages of moisture on the oven dry weight of soil, are given in figure 3. Examination of the results shows the variation in soil moisture that

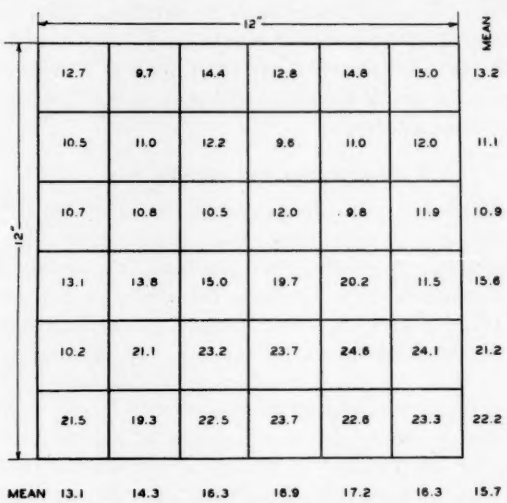

FIG. 3. Percentages of soil moisture within a 12-inch square on the face of a trench four feet from the trunk of a pear tree, in the central coast region of California. The samples were taken at the end of the growing season, the top row being 2½ feet below the surface.

occurred in samples taken a short distance from each other. In one case, a variation in moisture content of 12.5 per cent. occurred with a vertical distance of 2 inches. In general, it will be seen that the moisture contents as shown by the 12 samples taken from the bottom 4 inches on the face of the square with but few exceptions are considerably higher than those taken from the top 6 inches. The six samples taken 8 inches below the top edge of the square, in general, are intermediate between the groups just described. In many cases apparent irregularities in moisture contents may be caused solely by differences in soil texture. In such cases the irregularities may be reduced or eliminated by expressing the moisture content as relative wetness

(ratio between moisture content and moisture equivalent). In this case, plotting the results given in figure 3 on a basis of relative wetness, did not eliminate the variability. The soil under the trees was not of the same degree of wetness even at the end of the growing season. Since this variability was not caused by the texture of the soil, nor by uneven distribution of moisture because the plot received only rainfall, it follows that it was caused by the irregular distribution of roots. It is evident from these data that, although the orchard where these experiments were started was healthy and vigorous, and has been commercially profitable for many years, it was not suitable for experiments that require an accurate interpretation of the soil-moisture conditions existing during the growing season. The presence of layers of different kinds of soil beneath the surface, which prevented the uniform distribution of roots, was obscured by the method of securing the preliminary samples. These were obtained with a post hole auger that tended to pulverize and mix the different layers so that the samples secured appeared fairly homogeneous.

Discussion and summary

The data presented in this paper show the probable distribution of roots of certain deciduous fruit trees when grown on a reasonably uniform soil. The permanent wilting percentage was reached in a certain depth of soil close to the tree as soon as it was midway between adjacent trees. We have found a few cases, however, in which, because of the kind of root stock or the kind of soil, root distribution was uneven. In these exceptional cases, difficulty was encountered in interpreting the soil-moisture record. Under conditions of soil and root distribution similar to those in this series of experiments, reliance can be placed on a system of soil sampling during the growing season for the planning of a rational irrigation schedule.

Our experience has been that, if a system of soil sampling is to be used in accurately interpreting the plant-soil-moisture relationships during the growing season, the soil upon which the orchard is growing must be fairly uniform. If the soil is variable or if there is an uneven distribution of roots, irregularities in soil moisture of considerable magnitude are frequently found in successive samples. Relative wetness will not aid in interpreting results when root distribution is not uniform. However, when the irregularities in the soil-moisture record are caused by differences in soil texture, they may often be eliminated by using the relative wetness. Under conditions where the irregularities are caused by root distribution, it is better to plan future irrigations on the basis of observations either of the tree itself or of indicator plants growing in the orchard. The chief difficulty in using the response of deciduous trees or indicator plants is the suddenness with which wilting occurs. Ordinarily, the first signs of wilting, as indicated by

a drooping of the leaves, decreased rate of growth of fruit, or other plant responses, occur only a few days before the readily available water is exhausted. If the grower waits for the plants or trees to show visible signs of wilting before preparing for irrigation, the last trees to be irrigated, especially if the area to be watered is large and the irrigating stream is small, sometimes suffer from the lack of water. Regular soil sampling generally gives the grower a much longer period in which to prepare for irrigation, because he can predict with a reasonable degree of accuracy when irrigation will be necessary.

The fact that soil samples taken at any place within the experimental plots in mature peach, prune, and walnut orchards, which have had an even application of water, agree at comparable depths, shows that these trees, under the conditions existing at Davis, have a uniform distribution of roots. Samples taken at any position between the trees may be used to interpret the soil-moisture conditions.

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EFFECT OF SOIL MOISTURE ON THE RATE OF GROWTH OF LONGLEAF AND SLASH PINE SEEDLINGS

L. J. PESSIN
(WITH ONE FIGURE)

Introduction

Longleaf pine (*Pinus palustris* Miller) and slash pine (*P. caribaea* Morelet) are widely distributed in the Gulf Coastal Plain. MOHR (**12**) observed as early as 1896 that under natural conditions longleaf pine thrives on well-drained, sandy soils and that slash pine grows best on moist lands; also, MATTOON (**11**) found that slash pine is confined mainly to poorly drained lands and borders of swamps and rivers. Similarly PESSIN (**13**) found that longleaf pine inhabits both dry soils and moist soils but occurs most abundantly on well-drained soils, while slash pine grows principally in moist situations. In plantations, however, slash pine apparently grows as vigorously on the well-drained longleaf-pine lands as on moist soils, and young stands of slash pine under natural conditions sometimes establish themselves on well-drained soils some distance away from their parent trees. These new situations are quite different from those where the parent trees grow. This ability of the slash pine to grow on typical longleaf-pine sites suggests that both of these species may have similar moisture requirements, and that the restricted distribution of slash pine under natural conditions may be due not entirely to moisture conditions but also to other factors. A clear understanding of the moisture requirement of these species of pine is particularly essential for nursery practice, where the ultimate aim is the development of high-grade seedlings to insure high survival and good growth. Such a study as this should also throw light on the choice of suitable sites to plant to each of the species in the large planting program in the South, particularly in Louisiana, Mississippi, and Texas.

This study was undertaken to determine (1) the condition of soil moisture conducive to the best development of longleaf and slash pine seedlings; (2) the maximum and minimum amounts of soil moisture that each of these species can endure; and (3) the amount of dry matter produced by each species under different conditions of soil moisture. This last phase of the problem is essentially a study of the water requirement of these species of pines. By water requirement is meant the ratio of the total amount of water absorbed by the plant during a period of time to the amount of dry matter produced by the plant during that time. The water requirement of a plant is, therefore, a measure of the efficiency with which the water is used. This efficiency can be expressed by the formula $\frac{L}{D} = W$, where L indicates the total

weight of water lost from the plant through transpiration and evaporation; *D*, the total weight of the dry matter produced by the plant during the same period of time; and *W*, the number of units of water absorbed to produce one unit of dry matter. Inasmuch as the amount of water retained by the plant is relatively insignificant as compared with the amount of water lost, the amount of water transpired by the plant is taken as a measure of the water absorbed.

Review of literature

The problem of the efficiency of different species in their use of water has interested botanists, agronomists, and foresters for many years. IL'ENKOV (**8**) as early as 1865 studied the effect of soil moisture on trees. FITTBOGEN (**4**) found that the production of dry matter in oats was reduced greatly by the reduction of the moisture of the soil. VON HÖHNEL (**6, 7**) found that there was a definite relationship between the amount of leaf surface and the amount of water consumed by plants.

HELLRIEGEL (**5**) showed that the water utilization of plants decreased as the soil moisture content was lowered. MAERCKER (**10**) obtained similar results. VON DASZEWSKI (**3**) found that the dry weight of tubers was increased greatly in soil with high water content, while KIESSELBACH AND MONTGOMERY (**9**) found by improved methods that the reduction of the water content from 100 to 60 per cent. produced a reduction of the water requirement. In general, experiments on the effect of the soil moisture content on the water requirement of crop plants showed that the water requirements varied with the water content of the soil.

BRIGGS AND SHANTZ (**1**) found that field crops differed greatly as regards their efficiency in the use of water. Alfalfa, for example, used four times as much water as did millet to produce one pound of dry matter. In a later paper (**2**) they reported that even varieties of the same species differed widely in their water requirements.

Methods

In general, the method used in this work was similar to that used by some of the workers mentioned above. Plants were grown in sealed containers from April to October under greenhouse conditions. The total loss of water and the dry weight of the plants were calculated at the end of the experiment.

MOISTURE CONDITIONS

Five different moisture conditions were studied. Under *"very dry"* conditions the soil in each container received water amounting to 5 per cent. of the air-dry weight of the soil. Under *"dry"* conditions the water added to the soil amounted to 10 per cent. of the weight of the soil. Under *"damp"* conditions, water amounting to 15 per cent. of the weight of the soil was

added; and under *"moist"* conditions, 25 per cent. Under the *"wet"* conditions the soil of the longleaf pine received 35 per cent. of the air-dry weight of the soil, while that of the slash pine received 31 per cent. In both of these wet conditions the soil was saturated, and free water remained standing on the surface.

Inasmuch as it is impossible to maintain a constant moisture content in containers in which plants are growing, no such attempt was made in this study. What was actually studied was the amount of water within certain limits in each series. The uniformity of the distribution of moisture in the containers did not offer a serious problem, since the containers were small and the roots of the trees were well distributed within them.

Soil

The soil used in this study belonged to the Ruston fine sandy loam type. It was taken from the surface to a depth of 12 cm. on an area where the vegetation was composed mainly of longleaf pine saplings, species of *Andropogon*, and other piney-woods grasses and herbs. This soil was first passed through a coarse wire screen, then through a fine 2-mm. screen. This screened soil was spread out on canvas and allowed to dry for several days. Samples of the soil were then taken and the moisture content determined on an oven-dry basis. When the moisture content was less than 2 per cent., the soil was considered suitable for use. The soil used had an average moisture content of 1.18 per cent.

A physical analysis of the soil showed the following:

Soil fraction	Per cent.
Silt	26.1
Very fine sand	32.0
Fine sand	26.0
Sand	13.4
Coarse sand	2.5
	100.0

The colloidal content of the soil was 22 per cent. and the initial pH was 5.25.

Containers

Two kinds of containers were used. In the very dry, dry, damp, and moist series, paraffined paper milk bottles of 1-quart capacity were used. These were reinforced at the seams with adhesive tape and the entire surface of each was covered with a coat of waterproof shellac. On top of this, a coat of white enamel was applied. The mouth of each bottle was fitted with a paper milk-bottle cap with two holes to permit the passage of the stem of a plant and a glass tube, respectively.

In the wet series, mason jars were used. The mouths of these were closely fitted with paraffined screen-wire discs with two holes like those in the paper caps. Mason jars were used in this series because it was doubtful whether the paper containers could stand the heavy weight of water without becoming weakened and finally leaking.

The advantage in using paper containers was the facility with which the plants could be removed. After the completion of the experiment, the walls of the container were slit open with a sharp knife in several places and the containers placed in a receptacle. A fine stream of water applied to the slits soon loosened all the soil and the roots of the plants were removed without injury or loss of fine rootlets. Removing the roots from the glass jars took considerably longer than from the paper bottles.

Preparation of the cultures

Seeds of uniform weight were sown in flats in the same type of soil as that used in the containers. When the cotyledons were completely unfolded, each seedling was weighed and inserted through a hole in the cover of a container, which was then filled with the air-dry soil and weighed. The culture was then watered and allowed to stand until the seedling became well established. At this time the moisture in the culture was brought to the desired content. The lid of the container was then sealed with a mixture of paraffin and beeswax and the culture was again weighed. This final weight constituted the basic weight. Once every week all the cultures were weighed and the loss of water determined. Enough water was then added to bring the cultures back to the original basic weight. Fifty seedlings were used under each condition of soil moisture.

The seedlings were 41 days old at the beginning of the experiment, which lasted from April to the middle of October of the same year. The plants consequently were under observation during their first full growing season.

All the cultures were kept in the greenhouse, where the maximum temperature averaged 96° F. and the minimum 70° F. for the duration of the experiment. The relative humidity averaged 70 per cent. but sometimes reached a minimum of 49 per cent.

Analysis of results

The results are shown in table I. A statistical analysis of the data revealed a number of interesting facts. The longleaf pine seedlings showed best development in the moist soil, where the combined dry weight of the tops and roots was highest. The number of fascicles and length of needles were also greatest in the moist soil. Furthermore, in this soil the seedlings absorbed the most moisture, and the rate of transpiration was highest. This was due probably to the vigorous condition of the seedlings. The seedlings

TABLE I

SUMMARY OF EFFECT OF SOIL MOISTURE ON LONGLEAF AND SLASH PINE SEEDLINGS

Species	Soil moisture	Average amount of water absorbed		Average daily transpiration		Average number of fascicles		Average length of needles		Average length of taproot		Plants with more than 10 laterals	Plants with more than 10 mycorrhiza	Average dry weight				Water requirement	Average height of stem	Survival
														Tops (stems plus leaves)	Roots	Total (tops plus roots)				
		gm.	*s.e.**	*gm.*	*s.e*	*no.*	*s.e.*	*cm.*	*s.e.*	*cm.*	*s.e.*	%	%	*gm.*	*gm.*	*gm.*	*s.e.*	*gm.*	*cm.*	%
Pinus palustris	Very dry (5%)	505.97	53.4908	2.808	0.3108	6.5	0.3912	30.54	0.8017	26.88	1.1488	61	23	0.959	0.469	1.428	0.1020	354	0.5	98
	Dry (10%)	1,045.49	52.9532	5.292	0.3076	7.3	0.3833	28.21	0.8102	30.46	1.1372	58	46	1.043	0.669	1.712	0.1005	611	0.5	100
	Damp (15%)	1,384.80	53.4908	7.000	0.3108	8.8	0.3912	29.06	0.7936	32.82	1.1488	57	51	1.199	0.892	2.091	0.0120	662	0.5	98
	Moist (25%)	2,278.16	52.9532	11.992	0.3076	10.9	0.3872	34.79	0.7936	35.02	1.1372	82	94	1.902	1.714	3.616	0.1005	630	0.5	100
	Wet (35%)	1,026.54	54.6171	7.562	0.3175	8.9	0.3996	28.61	0.8102	42.62	1.1729	40	49	1.259	0.771	2.030	0.1039	506	0.5	94
Pinus caribaea	Very dry (5%)	373.82	57.1009	2.184	0.3318	7.4	0.4806	9.10	0.5892	26.46	0.7409	63	51	0.472	0.554	1.026	0.1086	364	8	86
	Dry (10%)	1,172.83	53.4908	6.216	0.3108	14.9	0.3792	13.29	0.4810	28.69	0.6941	82	73	1.011	1.270	2.281	0.1020	514	12	98
	Damp (15%)	1,666.39	53.4908	9.067	0.3108	16.4	0.3750	14.81	0.4707	30.89	0.6941	82	84	1.165	1.185	2.350	0.1020	709	12	98
	Moist (25%)	2,115.39	53.4908	12.563	0.3108	17.3	0.3672	15.18	0.4610	29.49	0.6941	86	96	1.331	1.081	2.412	0.1020	877	14	100
	Wet (35%)	1,038.66	56.4483	7.050	0.3281	9.3	0.4180	11.31	0.5167	26.75	0.7325	29	43	0.527	0.366	0.893	0.1072	1,163	10	98

* Standard error.

in this moist soil had the largest number of lateral roots and the largest number of mycorrhiza. The taproots were shorter than in the wet cultures but longer than in the other cultures.

The poorest development occurred in the very dry soil. Here the longleaf pine seedlings had the lowest dry weight, the fewest fascicles, the shortest taproots, and the fewest mycorrhiza. The seedlings in this soil had the lowest transpiration rate, and absorbed the least water. The needles were shorter than those in the moist soil but did not differ significantly from those growing in the other soils. The lateral roots were fewer than in the moist soil but more than in the wet soil.

The longleaf pine seedlings growing in the wet soil absorbed more water than did those growing in the very dry soil, about the same amount as those growing in the dry soil, and considerably less than those in the other two cultures. In this wet soil the seedlings transpired less than in the moist soil but more than those in the other cultures. The difference between the rate of transpiration in the seedlings in this soil and that of the seedlings in the damp soil was not very significant, and may be due to the fact that the two had the same number of fascicles. The seedlings in the wet soil had shorter needles than those in the very dry and in the moist soils. The taproots of the seedlings in this wet soil were longer than those in any of the other cultures, but the lateral roots were fewest. There were half as many mycorrhiza in the wet soil as in the moist, but twice as many as in the very dry soil. The dry weight of the seedlings in the wet soil was less than that of the seedlings in the moist soil, but higher than the dry weight of the seedlings in the very dry and dry soils.

The slash pine seedlings also absorbed the most water in the moist soil and had the highest transpiration rate in this soil. Also, the fascicles were more numerous and the needles were longer in the moist soil than in the other cultures. The taproots were shortest in the very dry and wet soils and longest in the damp and moist soils. The lateral and mycorrhizal roots were most numerous in the moist soil. The dry weight of the seedlings was highest in the moist soil, but did not differ significantly from that in the dry and damp soils. If the quality of the seedlings were to be judged by their dry weight, then the slash pine, unlike the longleaf pine, had no pronounced optimum condition, so that the seedlings appeared of about the same quality in the dry, damp, and moist soil, but were distinctly better than those in the extreme very dry and wet conditions.

In the wet soil the seedlings absorbed three times as much water as those growing in the very dry soil, but significantly less than those growing in the damp and moist soils. The transpiration rate in the wet soil was also three times higher than in the very dry soil, but considerably lower than in the damp and moist soil. The fascicles were slightly more numerous and the

needles were longer in the wet soil than in the very dry soil. The length of the taproot was about the same in both the wet and the very dry soils, but the number of lateral roots and mycorrhizal branches was considerably lower in the wet than in the very dry soil.

Discussion

The results (table I, fig. 1) indicate that both longleaf and slash pine seedlings showed best development in the soil which had an abundance of moisture but which was not completely saturated. In this moist soil the seedlings had a high transpiration rate and absorbed much water, with the result that here occurred the best development of the seedlings. Of interest also is the fact that the rate of transpiration was nearly the same for slash as for longleaf pine seedlings of the same age and in soils of similar moisture content. There also apparently exists a close relationship between the rate of transpiration and the moisture content of the soil; the transpiration rate was lowest in the very dry soil and highest in the moist soil, but in the wet soil it was low again.

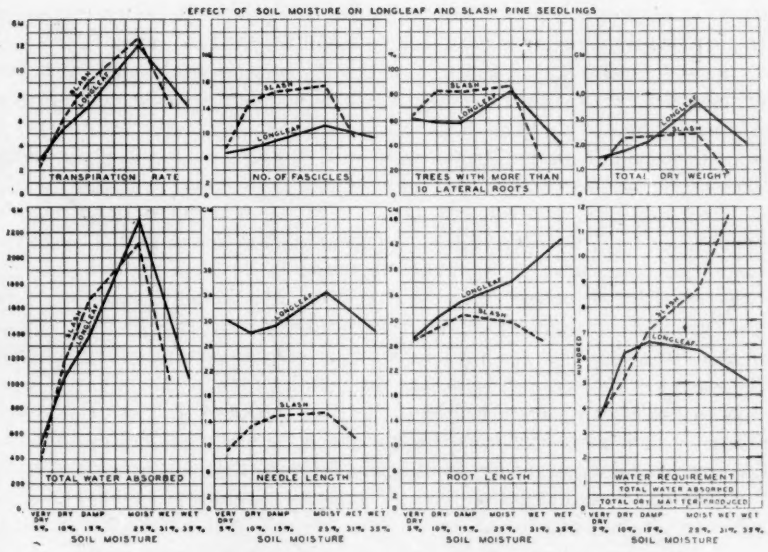

FIG. 1. Effect of soil moisture on longleaf and slash pine seedlings.

The commonly expressed idea that longleaf pine is a dry-land plant and that slash pine prefers wet sites is supported by the water requirement of the two species. In the early seedling stage, however, both species apparently

can endure extreme dry and extreme wet conditions. The mortality of slash pine in the very dry soil was higher than that of the longleaf in the same soil, and the mortality of longleaf was higher in the wet soil than that of the slash pine, but most of the seedlings of each species survived under the extreme conditions. The optimum moisture condition for growth is not markedly different for both species, but the slash pine seedlings apparently have a wider range of optimum moisture conditions than longleaf pine. The fact that under natural conditions longleaf pine is more abundant on dry sites and slash pine on wet sites may be caused by frequent fires which often do not seriously injure the longleaf pine seedlings, but sometimes annihilate whole stands of slash pine seedlings. The absence of longleaf pine seedlings on wet sites may be due not entirely to the wetness of the soil but to the dense vegetation shading out the longleaf pines and also to the fact that longleaf takes so long to start height growth. Slash pine seedlings apparently can endure considerably more shade than can longleaf pine seedlings.

Optimum moisture conditions are necessary for good development of the foliage of both longleaf and slash pine seedlings. Slash pine seedlings produced more fascicles than did the longleaf seedlings under all conditions, but the length of needles of the seedlings growing under optimum conditions (moist soil) was greater for the longleaf than for the slash pine.

The effect of soil moisture on root development showed some differences between the two species. In the wet soil the roots of the longleaf were considerably longer than those of the slash pine, but in the dry soil there was practically no difference. In the moist soil, however, the longleaf pine seedlings produced longer roots than those of the slash pine. The roots of the longleaf seedlings in the wet soil were over 50 per cent. longer than those in the very dry soil, while the slash pine roots in the wet soil and those in the very dry soil were about equal in length.

Of particular interest is the fact that in both longleaf and slash pine seedlings the roots of the most vigorous seedlings possessed the most mycorrhiza. The close relationship between the presence of mycorrhiza and the vigor of the pine seedlings is noteworthy and deserves further study.

The efficiency with which each of the pine seedlings utilizes the water is shown by their respective water requirements. For the sake of comparison, the water requirement of the seedlings in the very dry soil was expressed as 1 for both slash and longleaf pine. The ratios are shown in table II. It is evident that the efficiency of the pine seedlings in their utilization of water to produce dry matter differed not only with the moisture content of the soil but also with the species. In the very dry soil, both species were nearly alike in their water requirement, but with the increase in the moisture content the slash pine seedlings became less efficient; while the longleaf pine

TABLE II

RATIOS OF WATER REQUIREMENTS FOR LONGLEAF AND SLASH PINES IN SOILS OF VARYING MOISTURE CONTENT

SPECIES	VERY DRY	DRY	DAMP	MOIST	WET
Longleaf	1	1.7	1.9	1.8	1.4
Slash	1	1.4	1.9	2.4	3.1

seedlings showed no marked difference except in the wet soil, where the ratio was somewhat lower than in the other cultures.

A significant fact obtained in this study is that both longleaf and slash pine seedlings during their first year of growth apparently can endure very dry and wet soils. The longleaf pine can tolerate the dry conditions a little better than the slash pine seedlings, and the latter apparently can endure extreme wetness somewhat better than the longleaf; but the best conditions for growth apparently lie within the same limits of soil moisture for both species, the slash pine having a little wider range of favorable moisture condition. A soil that drains too rapidly or one that drains poorly apparently is not conducive to good growth and development of either species. In the light of this fact, it is possible to understand why slash pines growing on soils with underlying hardpan where the drainage is poor generally exhibit a stunted condition. Similarly, the absence of longleaf pine seedlings in savannas may also be caused by a condition of poor drainage. The savannas, which in many cases are old cut-over longleaf pine lands, possessed, before the removal of the trees, an upward drainage through the transpiration of the mature trees, but when these were cut, the soil (naturally poorly drained) became saturated owing to reduction of this upward drainage, making the site unfavorable for the establishment and growth of the longleaf pine seedlings.

The results of this study are particularly adaptable to nursery practice. If a watering schedule can be arranged in the nursery by which the optimum soil moisture can be maintained, a good grade of pine seedlings can be developed, thereby eliminating waste in culling.

Summary

1. Longleaf and slash pine seedlings were grown in cultures under different conditions of moisture including very dry, dry, damp, moist, and wet soils. In these the original total amount of moisture was brought back by frequent replacement of the moisture lost through transpiration.

2. The results indicate that longleaf pine seedlings during their first growing season exhibit the best growth in moist soils where the initial moisture amounts to about 25 per cent. of the total weight of the soil.

3. Slash pine is apparently not as exacting as longleaf pine in its moisture needs. No appreciable differences in growth are exhibited in cultures where the initial moisture ranges from 15 to 25 per cent. of the total weight of the soil. While both longleaf and slash pine seedlings, showed a high survival under extreme conditions of dryness and wetness, under these conditions they produced poorest growth.

4. Under moist conditions many mycorrhiza were produced, and all seedlings bearing mycorrhiza exhibited a particularly vigorous appearance.

5. The transpiration rates for both longleaf and slash pine seedlings were very similar; in both the rate varied with the amounts of moisture in the soil.

6. The water requirement generally was higher for slash pine under moist to wet conditions that for the longleaf pine seedlings under the same moisture conditions. The results indicate that slash pine seedlings require more moisture to build a unit of dry matter than do the longleaf pine seedlings.

SOUTHERN FOREST EXPERIMENT STATION
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TIME FACTOR CONCERNED WITH FLUIDITY CHANGES IN WARMED AND COOLED CELLS OF *ZYGNEMA* AND *SPIROGYRA*[1]

HENRY T. NORTHEN

(WITH THREE FIGURES)

Introduction

It is generally known that the viscosity of hydrophilic colloids changes during a long period after temperature equilibrium has been reached. BĚLEHRÁDEK (**1**) assumes that protoplasm responds to low temperatures as do hydrophilic colloids *in vitro,* and he suggests that the gradual slowing up of vital processes when cells are cooled to biological zero may be the result of a gradual increase in the viscosity of protoplasm. In other words, BĚLEHRÁDEK believes that the rate of diffusion, which in turn is dependent upon protoplasmic viscosity, may be slower than the rate of chemical change as such; and, hence, in a catenary series of reactions the rate may be limited by protoplasmic viscosity.

The data presented in this paper, however, indicate that when cells are warmed or cooled the viscosity of the protoplasm becomes constant when the temperature equilibrium is reached. Hence the fact that the rate of biological processes in cooled cells does not become constant when the temperature equilibrium is reached cannot be used as an argument in favor of the hypothesis that diffusion may limit the rate of a catenary series of reactions.

Materials and methods

For the determination of the time-factor when cells are cooled from 20° C. to 1° C., filaments of *Zygnema* and *Spirogyra* from water at 20° C. were laid between two strips of cotton which had been soaked in water at 20° C. The cotton wads containing the filaments were inserted in centrifuge tubes immersed in water maintained at 1° C. After the filaments had been in the ice water the desired time (tables II and V), they were centrifuged, still in ice water, with a hand-driven centrifuge. The filaments were then fixed and mounted in a 1 per cent. chromo-acetic solution and the distances the chloroplasts had been moved were determined. For *Zygnema,* the distances the chloroplasts had been moved were determined as previously described by NORTHEN (**2**). For *Spirogyra* no measurable criterion could be used, but the comparative displacements of the chloroplasts has been recorded by means of plus signs.

For the determination of the time-factor when cells are warmed from

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 163.

TABLE I

MINUTES OF EXPOSURE NECESSARY FOR THE FLUIDITY (DISTANCE CHLOROPLASTS MOVED) TO BECOME CONSTANT WHEN CELLS OF *Zygnema* WERE WARMED TO 20° C.

EXPERIMENT NUMBER	NUMBER OF CELLS MEASURED	AVERAGE LENGTH OF CELLS	FORCE × GRAVITY	SECONDS CENTRIFUGED	MINUTES IN WATER AT 20° C.	DISTANCE CHLOROPLASTS MOVED	σ	CELLS COLLECTED DURING
1	44	3.3	170	90	0.0	0.41 ± 0.024	0.234	September
	48	3.25			0.5	0.941 ± 0.02	0.202	"
	42	3.2			1.5	1.03 ± 0.014	0.134	"
	40	3.12			5.0	1.02 ± 0.01	0.126	"

1° C. to 20° C., filaments of *Zygnema* and *Spirogyra* were laid between two strips of cotton which had been soaked in water at 20° C. The cotton wads were inserted in the centrifuge tubes in water at 1° C. After the filaments had been in the ice water for ten minutes they were transferred to water at 20° C. After the filaments had been in the water the desired time (tables I and IV) they were centrifuged, mounted, fixed, and the displacements of the chloroplasts were determined as previously described.

To determine the approximate time necessary for a temperature equilibrium to be reached, a thermometer was laid between two strips of cotton soaked in water at 20° C. The cotton wad containing the bulb of the thermometer was then inserted in a centrifuge tube immersed in water at 1° C. The temperature was read at intervals of thirty seconds. A similar method was used to determine the time necessary for equilibrium to be reached when the temperature was raised from 1° C. to 20° C.

Data and conclusions

The approximate time necessary for temperature equilibrium to be established in the cotton wads is expressed graphically in figure 1. The results of the experimentation on *Zygnema* are recorded in tables I and II and in figure 2.

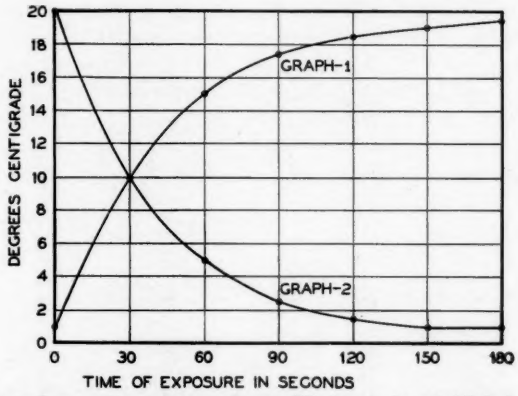

FIG. 1. Seconds exposure necessary for equilibrium to be established in cotton wads which were immersed in water at 20° C. (graph 1) or at 1° C. (graph 2).

In the preceding tables "length of cells" and "distance moved" are expressed in relative units—the units on the ocular micrometer which was used in measuring the displacements. The values may be converted into microns by multiplying by 34.75.

It will be observed, graph 1 of figure 2, that when cells are transferred from water at 1° C. to water at 20° C. the fluidity increases and becomes

TABLE II

MINUTES OF EXPOSURE NECESSARY FOR THE FLUIDITY (DISTANCE CHLOROPLASTS MOVED) TO BECOME CONSTANT WHEN CELLS OF *Zygnema* WERE COOLED TO 1° C.

EXPERIMENT	NUMBER OF CELLS MEASURED	AVERAGE LENGTH OF CELLS	FORCE × GRAVITY	SECONDS CENTRIFUGAL	MINUTES IN WATER AT 1° C.	DISTANCE CHLOROPLASTS MOVED	σ	CELLS COLLECTED DURING
2	54	3.35	170	35	3	0.701 ± 0.026	0.285	September
	47	3.27			6	0.609 ± 0.029	0.293	
	60	3.13			12	0.604 ± 0.017	0.205	
3	48	3.35		90	0	1.129 ± 0.026	0.265	
	48	3.5			3	0.74 ± 0.014	0.146	
	54	3.45			6	0.65 ± 0.028	0.301	"
	50	3.20			12	0.624 ± 0.024	0.253	
	51	3.22			24	0.625 ± 0.017	0.182	
	77	3.21			48	0.639 ± 0.017	0.218	
4	60	2.00		35	0	0.275 ± 0.008	0.097	
	52	1.99			2	0.10 ± 0.006	0.06	
	50	2.08			6	0.075 ± 0.004	0.065	October
	50	1.97			12	0.133 ± 0.007	0.078	
5	50	1.48			2	0.08 ± 0.006	0.059	"
	60	1.42			12	0.08 ± 0.003	0.029	
6	49	2.05		60	0	0.385 ± 0.009	0.08	
	52	1.92			3	0.14 ± 0.006	0.06	"
	50	1.92			6	0.19 ± 0.008	0.086	
	44	1.84			9	0.177 ± 0.004	0.047	
7	71	2.03		50	0	0.272 ± 0.008	0.097	
	63	2.13			1	0.244 ± 0.006	0.076	"
	60	2.10			3	0.161 ± 0.005	0.073	
	70	1.97			6	0.166 ± 0.006	0.073	
8	38	2.11	382	42	0	0.44 ± 0.009	0.079	
	42	2.08			1	0.300 ± 0.01	0.093	"
	38	2.03			2	0.171 ± 0.01	0.099	
	29	2.10			4	0.169 ± 0.009	0.069	
9	41	1.42			0	0.228 ± 0.005	0.051	
	57	1.43			1	0.122 ± 0.005	0.058	"
	54	1.40			2	0.09 ± 0.004	0.048	
	50	1.44			4	0.072 ± 0.004	0.044	

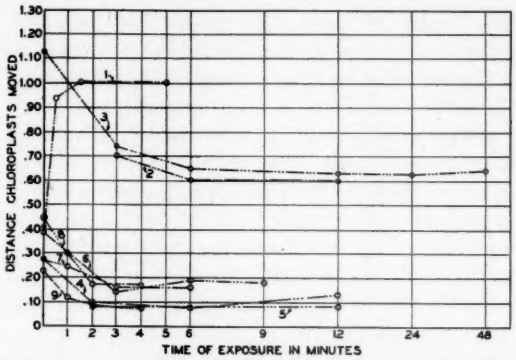

FIG. 2. Minutes exposure necessary for the fluidity (distance chloroplasts moved) to become constant when cells of *Zygnema* were warmed to 20° C. (graph 1) or cooled to 1° C. (graphs 2–9).

relatively constant within half a minute. An examination of graph 1 of figure 1 indicates that at the end of thirty seconds the probable temperature of the cells is only ten degrees. This indicates that the decided increase in fluidity occurs between 0° and 10° C. and that above 10° C. the increase in fluidity is much less marked. Moreover, the data indicate that when cells are warmed the fluidity becomes constant as rapidly as the temperature equilibrium is established.

Graphs 2 and 3 of figure 2, for long cells (hence, a greater distance to move), seems to indicate that the time necessary for the fluidity of the cytoplasm to become constant is more than three minutes and less than six minutes. However, graphs 4 to 9 (for shorter cells) indicate that the time

FIG. 3. Cells centrifuged for 45 seconds with a force of 680 × gravity. a, maintained in water at 20° C.; b, maintained in water at 1° C. for one minute; c, maintained in water at 1° C. for two minutes.

necessary for the fluidity to become constant is less than two minutes and more than one minute. In graphs 2 and 3 the higher fluidity at three minutes as contrasted with the fluidity at six or more minutes is probably spurious and may have resulted from a failure to stir vigorously enough the mixture of ice and water in which the filaments were immersed.

Graph 2 of figure 1 indicates that about two minutes are required for the temperature equilibrium to be established, and graphs 4 to 9 of figure 2 indicate that two minutes are required for the fluidity to reach a minimum. Hence, it becomes reasonably certain that as the temperature equilibrium is reached the fluidity becomes constant. In other words, the time factor for the fluidity to become constant when cells of *Zygnema* are warmed from 1° to 20° C. or cooled from 20° to 1° C. is zero.

The question next arises as whether or not phases more viscous than cytoplasm have a time factor of zero. In *Spirogyra* at least two factors determine the ease of displacing the chloroplast. First, the viscosity of the hyaline cytoplasm, and second, the ease with which the more viscous chloroplast can be bent. It is probable that the second factor is the limiting one, and hence, in *Spirogyra,* studies of displacements of chloroplasts give the time factor for the consistency of the more viscous chloroplast to become constant.

The results of the experimentation on *Spirogyra* are recorded in tables III, IV, and V and in figure 3.

TABLE III

RELATIVE DISPLACEMENTS OF THE CHLOROPLASTS AFTER TEN MINUTES EXPOSURE TO WATER AT TEMPERATURES OF 0°, 5°, 10°, 15°, AND 20° C.

TEMPERATURE	FORCE × GRAVITY	SECONDS CENTRIFUGED	RELATIVE DISPLACEMENT
0° C.	680	45	trace
5			++
10			++++
15			+++++
20			+++++

TABLE IV

RELATIVE DISPLACEMENTS OF CHLOROPLASTS IN CELLS OF *Spirogyra* MAINTAINED AT 1° C. FOR 10 MINUTES AND THEN MAINTAINED AT 20° C. FOR DIFFERENT PERIODS OF TIME

SECONDS OF EXPOSURE AT 20° C.	FORCE × GRAVITY	SECONDS CENTRIFUGED	RELATIVE DISPLACEMENT
0	680	45	trace
30			+++
60			+++++
90			+++++
120			+++++

TABLE V

RELATIVE DISPLACEMENTS OF CHLOROPLASTS IN CELLS OF *Spirogyra* MAINTAINED AT 20° C. THEN MAINTAINED AT 1° C. FOR DIFFERENT PERIODS OF TIME

SECONDS OF EXPOSURE AT 1° C.	FORCE × GRAVITY	SECONDS CENTRIFUGED	RELATIVE DISPLACEMENT
0	680	45	+++++
60			++
90			+
120			trace
150			trace
180			trace

The experiments recorded in tables III, IV, and V are representative ones. Each experiment has been repeated six times by the author or others in this laboratory and each time the same results have been obtained.

Graph 1 of figure 1 indicates that the probable temperature of the filaments after 60 seconds exposure was 15° C. It will be noted (table IV) that the displacement following sixty seconds exposure to water at 20° is the same as the displacement recorded in table III for material maintained at 15° C. At the end of ninety seconds the probable temperature of the filaments was 17.5° C. Upon comparing the value in table IV after ninety seconds exposure with the value at 15° or 20° C. in table III, it will be noted that the values are identical. Hence, in warmed cells of *Spirogyra* as well as in warmed cells of *Zygnema* the fluidity becomes constant as rapidly as the temperature equilibrium is reached.

If comparisons are made between graph 2 of figure 1 and tables III and V, it will be noted that when cells of *Spirogyra* are cooled from 20° C. to 1° C. the fluidity of the chloroplast becomes constant as rapidly as the temperature equilibrium is reached.

The experiments on *Zygnema* and *Spirogyra* demonstrate that when cells are warmed or cooled the consistency of the cytoplasm and the more viscous chloroplast becomes constant as rapidly as the temperature equilibrium is established. The time factor is zero, and hence it is doubtful that the gradual slowing up of vital processes when cells are cooled can be explained on the basis of a gradual increase in protoplasmic viscosity.

Summary

1. Filaments of *Zygnema* sp. and *Spirogyra* sp. were transferred from water at 20° C. to water at 1° C., centrifuged after various periods of time, and the distances the chloroplasts had moved were determined. Other filaments of *Zygnema* and *Spirogyra,* which were maintained at 1° C. for ten minutes, were transferred to water at 20° C., and after various time intervals were likewise centrifuged, and the displacements determined.

2. The results demonstrate that when cells are warmed to 20° C. or cooled to 1° C. the consistency of the protoplasm becomes constant as rapidly as the temperature equilibrium is established. The time factor is zero.

The writer takes pleasure in expressing his gratitude to Mr. MERRITT BODDY and to Mr. MICHAEL BARA of this department for aid in measuring displacements within cells.

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META- AND PYROPHOSPHATE WITHIN THE ALGAL CELL

ANNA L. SOMMER AND THOMAS E. BOOTH

Introduction

Comparatively little attention has been given to the pyro and almost none to the meta form of phosphate in plant and animal studies. Since reporting the occurrence of pyrophosphate in muscle in 1928, LOHMANN (**5, 6, 7, 8, 9, 10, 11, 12**) has conducted a number of experiments on the occurrence and transformation of pyrophosphate in animal tissue. He also made a few similar studies with yeast and plant material. Other work in which the pyro form of phosphate was considered was done by MEYERHOF and LOHMANN (**14**), BOYLAND (**1**), LEVITOV (**4**), EGGLETON and EGGLETON (**2**), EMBDEN, HEFTER, and LEHNARTZ (**3**), NEEDHAM and VAN HEYNINGEN (**15**), and ROCHE (**16**). According to LOHMANN (**7**) and BOYLAND (**1**), about one-fourth of the phosphate in yeast is in the pyro form. LEWITOW (**4**) reported that incubation of brewer's yeast did not alter the pyrophosphate content but that when this was done in the presence of glucose there was a considerable loss in pyrophosphate with a simultaneous decrease in orthophosphate. In most of the work, determinations for pyrophosphate might have included metaphosphate. WEISSFLOGG and MENGDEHL (**19**) found meta- and pyrophosphate in the roots of corn only when they used these forms as the source of phosphate in their culture media. Even in these cases they were unable to detect either meta- or pyrophosphate in the upper parts of the plant and concluded that these were converted into the ortho form before they reached the stems and leaves. Dr. RALPH MORGAN (private conversation) said that he believed metaphosphates were an important form in the living organism because they readily form complexes. An investigation was therefore undertaken to determine whether metaphosphate was present in living green plants and, if so, its relation to the presence of the ortho and pyro forms.

Experimentation

The ease with which meta- and pyrophosphates are converted to the ortho form makes it desirable to use, if possible, the intact living organism. The senior author and colleagues (**17, 18**) have shown that the ALLISON magneto-optic apparatus permits the direct detection of compounds within sufficiently small and transparent plants. The only requirement is that sufficient light be transmitted through the organism to give the minimum or minima of the compound being studied.

A pure culture of *Chlorella pyrenoidosa*[1] was used in most of the studies but similar results were obtained with other algae.

[1] The culture was obtained from Dr. D. APPLEMAN of the University of California at Los Angeles and was of a strain isolated by Dr. R. EMERSON of the California Institute of Technology.

Preliminary trials were made to determine if metaphosphate was present within the algal cell. A suspension of algae in a nutrient solution in which all of the necessary ions were present was placed in an observation tube and the minima for KPO_3 were sought. These were found to be present and angle readings[2] to within $\pm 0.5°$ were made. The suspensions were filtered through hardened filter paper, and readings were made on the filtrate. The filtrate usually showed no metaphosphate; and when the minima for this compound could be seen, the angle readings were very small.

After the preliminary tests showed the presence of metaphosphate within the algal cell, a study was made of its formation from the orthophosphate in the solution.

A culture solution was made up as follows:

$MgSO_4$	10	p.p.m.
$CaSO_4$	10	p.p.m.
KCl	12.5	p.p.m.
KNO_3	10	p.p.m. to 100 p.p.m.

A small amount of a heavy algal suspension from a culture in which all or nearly all of the phosphate had been utilized was added to this solution. Potassium acid phosphate was added to the suspension just before making the readings. Readings for K_3PO_4[3] and KPO_3 were made immediately after

[2] Angle readings vary directly with the concentration. BISHOP, E. R., DOLLINS, C. B., and OTTO, I. G. Magneto-optic rotation method for quantitative determination of calcium. Jour. Amer. Chem. Soc. 55: 4365–4370. 1933.

The data presented in table II were obtained by the senior author during the absence of the junior author. The readings, although much higher than those in table I, which the junior author observed, represent lower concentrations. Readings obtained by different observers may vary greatly for a given concentration as is shown in the following data.

COMPARISON OF ANGLE READINGS FOR THE SAME CONCENTRATION BY TWO OBSERVERS

OBSERVER	ANGLE READINGS IN DEGREES ($\pm 0.5°$) FOR CONCENTRATIONS OF						
	1×10^{-11}	1×10^{-10}	1×10^{-9}	1×10^{-8}	1×10^{-7}	1×10^{-6}	1×10^{-5}
Junior author	6.0	13.0	17.5	19.5	22.0	24.0	25.5
Senior author	18.5	34.5	46.5	65.5*	.....	.....	.....

* Readings above 60° are of very doubtful value.

The readings of the junior author are considerably lower and those of the senior author considerably higher than those obtained by most observers for similar concentrations. Readings for the same concentrations are usually quite constant for an individual over a considerable period of time but may vary over longer periods. Thus the readings of the junior author decreased gradually during a period of two years while those of the senior author increased greatly after eye strain from too constant observing. The data in table I were obtained at a time when a reading of 39° by the junior author corresponded to a concentration of about 1×10^{-4}.

[3] Readings for K_3PO_4 were made because it has been impossible to find minima for

preparing the suspension and again after the algae had been in contact with the solution for an hour or more. A final reading was usually made a day or two later.

It may be seen in table I that part of the orthophosphate was converted into the meta form. The final readings for the metaphosphate were usually higher than those for the orthophosphate when the relative amounts of algae

TABLE I

TRANSFORMATION OF ORTHO- TO METAPHOSPHATE WITHIN THE ALGAL CELL
(Angle readings vary directly with the concentration)

TEST NUMBER	ANGLE READINGS (± 0.5) IN DEGREES*					
	INITIAL		AFTER 1 TO 3 HOURS		AFTER 1 TO 2 DAYS	
	K_3PO_4	KPO_3	K_3PO_4	KPO_3	K_3PO_4	KPO_3
1	28.5	9.5	15.0	21.0	14.5	15.5
2	37.0	9.5	28.5	14.5	16.0	18.5
3	27.0	8.0	17.0	16.5	.....	.....
4	22.0	8.0	7.5	14.5	6.0	8.0
5	22.0	7.5	12.0	13.0	9.0	16.5
6	20.0	9.0	12.5	18.0	5.5	16.5
7	31.5	9.0	29.5	12.5	26.5	12.0
8	36.5	12.5	33.0	13.5	26.0	9.5
9	28.5	12.0	23.5	13.0	23.0	9.0

* Observations by junior author. See footnote page 200.

and phosphate were such that the phosphate was rapidly disappearing. When, however, the amount of phosphate as compared with the algae was greater, the amount of metaphosphate appeared to hold a fairly constant level and the orthophosphate disappeared more slowly.

A second study was made to determine if pyrophosphate appeared as an intermediate form between the ortho- and metaphosphates. Algae used for this work had been growing for a long period without the addition of phosphate to the culture solutions. Suspensions were made in solutions of the composition previously stated. Examinations of the suspensions showed no ortho- or pyrophosphate. Metaphosphate was present in the suspensions made up on the first two days but it could not be detected in those made up later. Three aliquots of the suspension, two with algae and one from which the algae had been filtered were used each time. Orthophosphate was added to one of the unfiltered aliquots and to the filtered aliquot. Examinations were made before and just after the addition of the phosphate, and again after an hour or more. Final readings were usually made the following day.

salts of HPO_4^{--} or $H_2PO_4^{-}$. When such salts are dissolved, minima are obtained for the salt of PO_4^{---} (the amount of phosphate determined being equivalent to the amount of the particular cation present unless this cation had been added in excess of the phosphate), the hydroxide of the cation, and H_3PO_4. In this work an excess of the cation of the salt investigated was always present.

TABLE II

RELATIONSHIP OF ORTHO-, PYRO-, AND METAPHOSPHATES WITHIN THE ALGAL CELL
(ANGLE READINGS VARY DIRECTLY WITH THE CONCENTRATION)

TEST NUMBER	TYPE OF SOLUTION	ANGLE (± 0.5) READINGS IN DEGREES*								
		INITIAL			AFTER 1 HOUR			AFTER 2 OR MORE HOURS		
		PO_4^{---}	$P_2O_7^{----}$	PO_3^-	PO_4^{---}	$P_2O_7^{----}$	PO_3^-	PO_4^{---}	$P_2O_7^{----}$	PO_3^-
1	Without PO_4	0-†	0-	43.5	0-	0-	43.5	.....	.....	.....
	With PO_4	44+	0-	43.5	+‡	14.5	46.0	.....	.....	.....
	With PO_4 (filtered)	44+	0-	0-	+‡	0-	0-	.....	.....	.....
								After 2 hours		
2	Without PO_4	0-	0.5§	19.5	0-	0-	19.5	0–	0–	19.5
	With PO_4	59.5	0-	19.5	57.5	26.0	24.0	56.5	27.0	29.5
	With PO_4 (filtered)	59.5	0-	0-	59.5	0-	0-	59.5	0–	0–
								After 24 hours		
3	Without PO_4	0-	0-	0-	0-	0-	0-	0–	0–	0–
	With PO_4	60.5	0-	0-	59.5	11.0	10.0	55.0	25.5	44.5
	With PO_4 (filtered)	60.5	0-	0-	60.5	0-	0-	60.0	0–	0–
4	Without PO_4	0-	0-	0-	0-	0-	0-	0–	0–	0–
	With PO_4	60+	0-	0-	60+	20.0	20.0	60+	46.0	50.0
	With PO_4 (filtered)	60+	0-	0-	60+	0-	0-	60+	0–	0–

* Observations by senior author. See footnote page 200.
† 0-, minima not seen at 0°.
‡ With PO_4 present but angle reading not determined.
§ Probably contamination in tube.

Pyrophosphate soon appeared after the addition of orthophosphate to the suspensions and persisted over the period in which the determinations were made. The amount present shortly after the addition of the orthophosphate was sometimes greater than that of the metaphosphate, but later determinations always showed an amount of metaphosphate greater than that of the pyrophosphate. The results of these tests are shown in table II.

Discussion

The transformation of orthophosphate to the meta form and the persistence of metaphosphate after the ortho and pyro forms could no longer be detected indicates that the meta form is important in the metabolic processes of algae. Some of the phosphate in biological material determined by certain investigators as pyrophosphate may have originated from the meta form since their methods did not distinguish between the two. According to WEISSFLOG and MENGDEHL (**19**), meta- and pyrophosphates are rapidly transformed into the ortho form after they are absorbed by corn plants. The fact that they found no meta- or pyrophosphate in the stems and leaves may have been because the amounts were too small to be determined by their method. MENGDEHL (**13**) was able to determine meta- and pyrophosphate which he added to plant material. It seems, therefore, unlikely that if considerable amounts were present in the material from corn plants used by WEISSFLOG and MENGDEHL (**19**) they would have been converted to the ortho form by the time the determinations were made. The results in table II show an amount of pyrophosphate greater than that of the meta form only for a time after the orthophosphate had been added to cultures deficient in phosphate. Algae grown for several months with adequate phosphate and then suspended in a solution of the composition given above also showed metaphosphate to be present in greater amounts than the pyrophosphate. The circle readings were as follows: $Mg_3(PO_4)_2$, 50°[4]; $Mg_2P_2O_7$, 26.5°; and $Mg(PO_3)_2$, 41.5°. Examinations of the solution before the addition of the algae showed no meta-, ortho-, or pyrophosphate. These results indicate that pyrophosphate might be present only as an intermediate step between the ortho and meta forms.

Summary

Living algal cells were examined by means of the ALLISON magneto-optic apparatus for the presence of meta- and pyrophosphates. These were found to be present except where the algae had been in phosphate-deficient media for a long time. Metaphosphate was present after the ortho and pyro forms could no longer be detected. The transformation of ortho- to metaphosphate

[4] Readings not made above 51°.

and the persistence of the metaphosphate indicates that this form is important in the metabolic processes of algae.

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BRIEF PAPERS

PREPARATION OF INULIN FOR USE IN ADSORPTION COLUMNS

H. A. SPOEHR

Inulin has been found to be a very satisfactory material for the separation of the two chlorophyll components by means of the chromatographic adsorption method of TSWETT. Inulin has certain advantages over powdered sucrose for this purpose: it is less hygroscopic than sucrose; it apparently has a higher adsorptive capacity, so that smaller quantities and hence smaller columns are needed; the chlorophyll separates into two bands of the two components readily and these are easily eluted. Moreover, it is not necessary to remove the carotenoid pigments from the solution before adsorption, so that leaf extracts in petroleum ether solution can be used directly on the inulin adsorption columns.

The method which has been used for the preparation of inulin is in principle the same as that described by KILIANI (**1**) and which has recently been used by SPOEHR and MILNER (**2, 3**) for the isolation of leaf starch from plant materials. The source for the preparation of inulin has been dahlia tubers which were obtained as culls from nurseries.

The dahlia tubers were scrubbed to remove all soil, and decayed and woody portions were cut out. The tubers were then cut into slices of a convenient size to feed into a mincer through which they were passed three or four times, using a medium-sized cutter. This was found to be better than to mince the material very fine, because of the loss of juice with the fine mincer. Batches of five kilos of the ground tubers, mixed with three liters of water and 25 gm. of powdered calcium carbonate were boiled vigorously for one-half hour. The hot mixture was filtered with suction through muslin on a large Buchner funnel and the pulp thoroughly pressed out on the funnel. The pulp in the funnel was then washed with 500 cc. of hot water and thoroughly drained by suction and pressure. A second extraction of the pulp hardly repays the time and effort required. The hot filtrates were immediately filtered again through large folded filters to produce a perfectly clear, dark brown solution.

The solutions obtained in the manner just described were then subjected to freezing. This was done in five-gallon ice cream cans, which were stored at $-20°$ C. for 5 days. The solutions were then allowed to thaw at room temperature. The inulin remained insoluble and was removed by filtration through filter paper on a Buchner funnel under reduced pressure, or preferably on large sintered Jena glass filters. The phenomenon is very similar to that of retrogradation of starch (**2, 3**). Incidentally it may be mentioned that it has been found impossible to separate glycogen from aqueous solution

by this method. The filtration of the inulin may become a rather tedious process and it was found advantageous to filter the main portion with only slightly reduced pressure in order to avoid clogging the pores of the filter. But if the inulin solution has been kept frozen for some days and the filtration is done cautiously, it proceeds fairly rapidly. The moist inulin is very susceptible to infection by molds, so that it should be worked up immediately.

The inulin was then dissolved again in hot water, filtered, and the solution frozen at – 8° C. The volume of solution in this step was very materially less, about one-tenth of that used in the first extraction, and the freezing process was carried out in ice trays of a household electric refrigerator. After several days, the solutions were allowed to thaw and the inulin was filtered on sintered glass filters with suction, with the precautions already mentioned. The inulin was dried in an oven at 60°, coarsely ground, and dried in vacuum over calcium chloride for several days. It was then powdered in a pebble mill, passed through a 200-mesh sieve, and dried in vacuum over phosphorus pentoxide.

The inulin thus prepared was pure white; different preparations had an ash content of 0.16 to 0.20 per cent. and a specific rotation of $[\alpha]_D^{20} = -35.3°$ to – 38.1°. From one lot of 27.8 kilos of dahlia tubers, which were extracted in six batches, there were obtained first crops totaling 1640 gm. of inulin, *i.e.*, a yield of 5.8 per cent. The filtrates from the second freezing were combined, concentrated, decolorized with charcoal and frozen again, and yielded an additional 85 gm. of inulin, making a total yield of 6 per cent.

One further advantage of the use of inulin is that it can be readily recovered after use as an adsorbent. It is washed with ethanol, dissolved in hot water, filtered, decolorized with charcoal, if necessary, and by freezing the solution it can be recovered in pure form as described.

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EFFECTS OF X-RAYS ON SEEDS

A. A. Bless

In a recent paper (**1**) the author has shown that seeds subjected to various doses of x-rays show peculiarities at certain stages of the growth which are not necessarily maintained throughout the period of growth, and cannot be used as a criterion for the determination of the best dosage for yield at maturity.

In order to obtain some information concerning the effects of x-rays on seeds, by examination of mature plants from x-ray treated seeds, experiments were made on seeds of radishes, lettuce, and beans. The x-ray tube was operated at about 100,000 volts and 5 milliamperes. The rays were filtered through 1 mm. of aluminum, and the seeds were placed at a distance of 30 cm. from the target. They were subjected to the radiation for various lengths of time. The growth of the seeds was recorded every week, and showed phenomena similar to those exhibited by corn in the paper mentioned above and in agreement with the work of Johnson (**2**) and will not be discussed here.

According to Shull and Mitchell (**3**) the effects due to the x-rays are more pronounced when the seeds are subjected to the radiation in sprouted condition. In order to test this hypothesis two sets of experiments were conducted; one set of seeds was exposed to x-rays when dry, while the other set was exposed when the seeds were sprouted. For the latter set the seeds were allowed to remain in trays on sterilized cotton padding saturated with distilled water until definite sprouts appeared and then subjected to x-ray treatment. The results of the treatment are shown in table I.

The results show that the x-ray treatment did not produce significant changes in the case of radishes. The improvements in weight of the plants from treated seeds as compared with those from untreated is not very large, and may therefore be attributed to accident. Moreover there is no significant difference between the weight per pod of the seeds exposed when sprouted and those exposed when dry. The increase on the average is only about 10 per cent. which cannot be considered to be very important.

In the case of beans, plants from seeds treated dry have shown no significant changes over the untreated. However, plants from the irradiated sprouted seeds have shown increases of some 25 per cent. over the controls, which is probably too large to be considered accidental.

The results were more interesting in the case of lettuce. While the increase in weight was not significant in the case of most of the plants produced from seeds treated in the dry state, the five-minute dose produced an increase in growth of about 60 per cent. over the controls. Because of this large increase, the experiments were repeated a year later. The results indicated

TABLE I

Plant	Time of exposure	Exposed dry — Weight per plant		Exposed sprouted — Weight per plant		Remarks
	min.	*gm.*	*gm.*	*gm.*	*gm.*	
Radishes, (Cincinnati Market)	0	3.3		4.0		In all 250 plants; about 20 plants in each set
	¼	4.3		5.3		
	½	2.0		2.4		
	¾	5.3		4.8		
	1	3.5		4.5		
	2	4.8		5.7		
	3	2.7				
	4	4.5		4.4		
	7	4.5				
Beans (Bountiful)	0	6.2		5.3		300 pods; about 25 in each set
	¼	4.8		3.8		
	½	5.1		8.2		
	1	6.4		6.9		
	2	6.2		5.3*		
	4			7.3		
	6			5.5*		
		1936	1937	1936	1937	
Lettuce (White Boston)	0	4.3	4.0	4.3	5.0	300 plants; about 10 in each set
	¼	4.7		6.0		
	½	3.7		3.8*		
	¾	3.9		2.0*		
	1	4.7	4.0	5.1	6.9	
	2	4.0	6.65	5.8	5.7	
	3	3.6	4.5	4.7		
	4		6.0		8.6	
	5	7.0	6.1		7.7	
	6		4.9	5.0	6.1	
	7	3.4	4.7		8.1	

* Poorly sprouted.

that for the 4- and 5-minute exposures the increase is well over 25 per cent. In the case of the seeds irradiated when in sprouted condition, the increase in weight of plants grown from the treated seed over the controls was on the average about 50 per cent. for the larger doses used.

In addition to the differences in weight the x-rays seem to have also caused a noticeable difference in taste. About half of the people who have tasted the lettuce in 1936 have been able to detect this difference. The lettuce seemed to have a stronger, "tangier" taste, the "tanginess" increasing with the dose. In 1937 eight people out of twelve who tasted the lettuce have observed this difference. Considering the fact that the sense of taste varies a great deal with different individuals, the difference introduced by x-rays is probably real.

In addition to these greenhouse experiments, x-rayed seeds of corn were planted in the field. For these experiments, an inbred variety developed

in the Florida Agricultural Experiment Station was used. The seeds were planted in the field and so staggered as to minimize the effects of possible soil variation. The inbred variety, while possessing the advantage of small individual variation, possessed the great disadvantage of lower resistance to diseases and to the ravages of the corn borer. In place of the two hundred ears of corn expected for each batch, only about 40 per cent. of the normal crop was obtained. The results are presented in tabulated form.

Time of exposure in minutes (exposed dry)	Weight in grams per ear
0	204
1	213
2	198
4	213
8	212

It appears that for two or three doses, there is a small increase in weight of the ears grown from the x-rayed seeds over the controls. The result cannot be considered very significant.

The results of these experiments may be summarized briefly as follows: within the limits of the x-ray doses used in these experiments, significant effects of x-ray on the yield at maturity have been found only for lettuce and for beans. The response to the x-ray treatment was greater when the seeds were in sprouted condition. No significant changes have been observed in the case of radishes, exposed sprouted as well as dry, and of corn exposed dry.

It gives me great pleasure to acknowledge the advice and the generous cooperation which has been given to me by Dr. F. S. Jamieson and Dr. F. H. Hull, of the Florida Experiment Station.

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NOTES

Indianapolis Meeting.—In many ways the fourteenth annual meeting of the American Society of Plant Physiologists was the most satisfactory meeting in its history. The arrangements left little to be desired, and more was accomplished for the future welfare of plant physiology than at any previous meeting. Space does not permit publication of details of the actions taken, but they will vitally affect the financial background of the Society's program. The program committee, through the efforts of its chairman, Mr. WITHROW, and with the able assistance of all members of the committee, developed a series of meetings that set a high standard of excellence. The annual dinner was attended by about 175 individuals. As usual, the important features of this gathering were the STEPHEN HALES address, *Hormones and the Analysis of Growth,* by Dr. K. V. THIMANN, and the announcements of awards and honors. The officers of the Society, and its committees responsible for the success of the meeting, deserve great credit for the high standards set in the performance of their duties. We are indeed fortunate in having so many capable and efficient servants.

Annual Meeting, 1938.—The next annual meeting of the Society will be held at Richmond, Virginia, in connection with the A.A.A.S. meetings in December, 1938. The personnel of the program committee, as well as that of the program committee for the summer meeting at Ottawa, Canada, late in June, will be announced in April. Early consideration of symposia subjects and joint meetings is advisable so that more careful preparation can be made by those participating in the discussions. In addition, careful consideration should be given in advance to such problems as hotel and meeting room requirements in order that the most favorable arrangements may be made when the assignments are in order.

Life Membership Awards.—The BARNES Life Membership committee had been authorized to make two awards at Indianapolis, if it was found feasible to do so. In accordance with this authorization, which followed a precedent set at the Des Moines meeting in 1929, the committee, with Dr. H. R. KRAYBILL as chairman, presented the names of two candidates for the awards. The two men chosen for this high honor were Dr. HOMER LEROY SHANTZ, of the U. S. Department of Agriculture, and Dr. ALEXANDER P. ANDERSON, Tower View Laboratory, Red Wing, Minnesota. Brief biographies of the two candidates are given.

Dr. A. P. ANDERSON was born in Red Wing, Minnesota, on November 22, 1862. His collegiate training was obtained at Minnesota, where he received the B.S. degree in 1894, and the M.S. degree in 1895. As was customary at

the time, he went to Germany for his Ph.D., which was granted at Munich in 1897. He was botanist and bacteriologist at Clemson College just after his return from Europe, assistant professor of botany at Minnesota for two years, once more biologist and entomologist at Clemson during 1900–1901, and curator of the herbarium at Columbia University for a short period. After this he went into private research, which took an industrial turn, and to this work he has given a lifetime of service. He was included among the first group of 100 botanists to be starred in the first edition of *American Men of Science.*

His father, JOHN ANDERSON, and his mother BRITTA (GUSTAFSDOTTER) ANDERSON, were both natives of Sweden. He married LYDIA JOHNSON, of Highlands, North Carolina, shortly after returning from Munich. Five children have graced their home.

Dr. ANDERSON is best known for his invention of puffed cereals, which came out of his early work on processing of starch and cereal products. Puffed rice, puffed wheat, Quaker crackels, and other puffed products we owe to his inventive genius. But before he went into these industrial problems, he was interested in the formation of abnormal resin canals in the Coniferae, in the growth of fruits as determined by weighing, and in plant diseases. He has on various occasions shown his interest in the work of the American Society of Plant Physiologists, as a contributor to the STEPHEN HALES fund in 1927, and as a patron of the Society. He passed his 75th birthday anniversary in November, 1937. The action of the committee in making the award is very appropriate.

Dr. H. L. SHANTZ was born in Kent Co., Michigan, on January 24, 1876. He received the B.S. degree from Colorado College in 1901, and the Ph.D. degree from the University of Nebraska, in 1905. He had had experience in the teaching of botany at Colorado College, and at Nebraska, during his graduate training. After obtaining his doctorate, he served for short periods at Missouri, and at Louisiana. In 1908 he joined the staff of the U. S. Department of Agriculture, and was especially interested in the problems of alkali and drought resistance, and the breeding of resistant races. He was plant physiologist in the Bureau of Plant Industry from 1910 to 1926, and in charge of plant physiology and plant geography during the last six years of this period. During 1926 to 1928 he was Professor and head of the Department of Botany at Illinois, from which institution he went to the University of Arizona as president in 1928. Recently he has returned to the Department of Agriculture.

In 1901 Dr. SHANTZ was married to LUCIA MOORE SOPER. They have two sons. In connection with his varied services, Dr. SHANTZ was a traveler and explorer. He went with the Smithsonian Expedition to Africa in 1919–1920, and was a member of the Educational Commission to East Africa under the Phelps Stokes Fund and the International Educational Board in 1924.

His name is best known among plant physiologists and ecologists for his work on the water relations of plants and soils, the wilting coefficient, the moisture equivalent, the water requirement of plants, etc., and for his studies of the indicator value of native vegetation in the Great Plains area. These investigations, however, represent but a small part of his scientific contributions which have been many and varied.

The American Society of Plant Physiologists is greatly honored by the addition of the names of these two eminent men to its roll of CHARLES REID BARNES life memberships.

Corresponding Members.—During the year 1937 two corresponding members of the American Society of Plant Physiologists were lost by death. At the Indianapolis meeting, therefore, two new members were elected. The two plant physiologists chosen for these honors were Dr. HENRY H. DIXON, of Trinity College, Dublin, Ireland, known throughout the world for his contributions to the problems of ascent of sap, particularly for the cohesion theory of sap rise, and Dr. ALFRED URSPRUNG, of Fribourg, Switzerland, known best for his accurate measurements of osmotic pressure. To these men American plant physiologists extend cordial greetings and best wishes. The Society is happy to honor these distinguished men for their great contributions to plant physiology.

New England Section.—The New England Section is planning its annual spring meeting for May 3 and 4, 1938. The meeting is to be held at Orono, Maine, under the auspices of the University of Maine. In addition to a session for short papers for Friday afternoon, May 3, there will be a symposium on the physiology of aquatic plants. Participants in this symposium will be Dr. F. H. STEINMETZ and Dr. G. P. STEINBAUER, of the University of Maine, Dr. W. C. MUENSCHER, Cornell University, and Mr. CLARENCE COTTAM, of the U. S. Biological Survey. The annual dinner will be held as usual on Friday evening, with after dinner discussions. These New England Section meetings have been very well attended in the past, and it is hoped that all members of the section will plan to attend this meeting at Orono.

Endowments.—The plans which have been developing over a number of years for the gradual increase of the general endowment of the American Society of Plant Physiologists have now been completed and put into operation. The final steps were taken at Indianapolis. In a few years it will be possible to estimate the average productivity of the plan as a whole. During 1937 the general endowment was enlarged from somewhat over $700 to about $1200. Much of this growth was obtained through gifts of patrons, but there will be steady growth in the future due to other sources of endowment

income. It is hoped that the steady increase in the number of patrons may continue. Is there any more enduring benefit to mankind which one can leave behind him than the possibility of continuously increasing knowledge? Those who become patrons are helping to make more certain the perpetual enrichment of human life through scientific investigation. Gifts are always carefully safeguarded, and the need challenges conscientious thought on the part of all scientists as well as laymen.

Patrons.—At the Atlantic City meeting in 1936 the first patrons of the Society were elected. Two more were added at the Denver meeting in June, 1937, and another was added at Indianapolis. The latest addition to the ranks is Professor FRANK M. ANDREWS, for many years plant physiologist at Indiana University. It is gratifying to know that the aims and purposes of our organization are appealing to so many people in terms of permanent support. Professor ANDREWS has conferred a great honor upon the Society by this additional evidence of his loyalty, devotion, and generosity toward it, a loyalty and devotion which has been expressed in many other ways during the last 15 years. It is a pleasure to record, and to express to him our appreciation of his generous aid.

Bohumil Němec.—During the last year we have attempted to bring closer to American workers the great personalities of our corresponding members. In this number we pay our tribute of honor and respect to Dr. BOHUMIL NĚMEC, who reaches his 65th birthday anniversary on March 12, 1938. On behalf of all American plant physiologists, we extend to him our cordial greetings and good wishes for that happy occasion, and wish him long years of usefulness in the field to which he has contributed so ably. Inasmuch as the story of the progress of plant physiology in Czechoslovakia is so closely identified with the life of Professor NĚMEC, we are not attempting any additional biographical sketch at this time. We are greatly indebted to Dr. PRÁT for his assistance with the photograph which is reproduced in this issue of PLANT PHYSIOLOGY, and to Professor FRANK M. ANDREWS through whose efforts the original was rephotographed to improve the reproduction.

Hans Molisch.—Dr. HANS MOLISCH was born July 12, 1856, and was therefore 81 years old at the time of his death on December 8, 1937. For a good many years he had been professor of botany at the University of Vienna. He had also been professor of botany at Praha and at Sendai, Japan. A good part of his research has been done in the United States, in Java, and also in India. At the University of Praha he was director of the Plant Physiological

Institute and later also held the same position in the University of Vienna. He was the author of about 200 scientific contributions many of which were in the form of books. It would take us too far to enumerate the volumes he has written. He always wrote in a very interesting way and with remarkable clearness. He succeeded WIESNER and during the last 50 years he has been busily engaged in his scientific investigations. His plant physiological investigations have influenced almost every phase of this science. Many of his books are absolutely indispensable to the plant physiologist. Among these may be mentioned his *Mikrochemie der Pflanzen* which covers a field in which he made many valuable contributions. His correspondence was very large but nevertheless he, as was true of many other great men, always found time to write his letters in long-hand. Professor MOLISCH travelled extensively and wrote several volumes concerning these travels. He was a keen observer and always wrote just as interestingly of his travels as of his direct investigations. On one of these trips he visited the United States. Professor MOLISCH has been the recipient of many honors; during his 80th year he was vice-president of the Academy of Science of Vienna, and at the time of his birthday was highly honored and received distinction from many countries. In 1935 Professor MOLISCH was elected a corresponding member of the American Society of Plant Physiologists. Those who knew and read after Professor MOLISCH will miss him greatly. He was one of the most famous plant physiologists and in his death science has suffered an irreparable loss.—Memorial Committee.

Upendra Kumar Das.—Plant physiology has suffered another serious loss in the death of Dr. U. K. DAS. He was born in Habiganj (Assam), India, July 22, 1902. He attended the University of Hawaii where he received the B.S. degree with honors in 1927 and the M.S. in 1930. From 1927 to 1934 he was Research Agriculturist in the Hawaiian Agricultural Experiment Station at Honolulu. He received the Ph.D. degree from the University of Minnesota in December, 1935. Although just beginning his research work his publications number 15 in the Hawaiian Sugar Planters' Record. His thesis on *Nitrogen Nutrition of Sugar Cane* was published in PLANT PHYSIOLOGY **11**: 251–317. 1936. Due to an explosion of apparatus in his laboratory he was instantly killed on October 22, 1937. He possessed a thoroughly scientific attitude of mind and was eminently practiced in all his work. He will be greatly missed by all of his associates and others as well. His death is a great loss to plant physiology and to replace him will be difficult.—Memorial Committee.

Chronica Botanica.—This chronicle of botanical progress has been reorganized, and becomes a bi-monthly journal instead of a year-book with the

issues of 1938. The subscription price has been reduced from 15 to 7 guilders. The aims of the new journal continue those of the former yearbook, with the addition of publication of brief papers and discussions. Its purposes are to promote documentation, good will, and international cooperation among plant scientists. There are eight other sections provided in the new journal as follows: scientific communications, forum botanicorum, international congresses, quotations, miscellaneous news, personalia, queries, and new periodicals. Those interested in possession of the new journal, individuals or librarians, or those who wish to use its facilities in the publication of scientific notes, discussion, or news are invited to correspond with FR. VERDOORN, P.O. Box 8, Leiden, Holland.

Agrobiology.—*The ABC of Agrobiology* is the title of a recent book by O. W. WILLCOX, who has written several works in which he intimates that agriculture, pursued in the fulfillment of certain mathematical equations, would be enormously more productive than it is now. There are 18 chapters in this new venture, enough, almost, for an A to Z presentation.

If all limiting factors could be lifted from plant growth, and if the growth capacity of all agrotypes could be built up to theoretical maxima by genetic improvement, of course we would have larger yields. But this would require perfect climatic conditions, as well as perfect soil conditions, both of which are unattainable in any very considerable area under cultivation. Mathematical equations look like accuracy and precision, but they do not help one if he lives in a dust bowl! Furthermore, WILLCOX assumes that the percentage of nitrogen which plants will build into their bodies is genetically controlled and constant for any given agrotype. He figures out that no plant type can take over 318 pounds of nitrogen from an acre of soil. By dividing percentage of nitrogen content into this value, he computes theoretically possible yields.

Against these assumptions, one must place the known facts that one can vary the amount and kind of nitrogen present in plants almost at will; that there is no constancy in the nitrogen relations; that maximum nitrogen intake often means decidedly low yielding capacity; that the quality of fruits and vegetables may be ruined by too much nitrogen, etc. The experienced grower will have no difficulty in detecting many of the errors involved in WILLCOX'S reasoning.

The author evidently intended this book to be elementary enough to assist the lay-gardener, orchardist, and general farmer. Unfortunately there does not seem to be much chance that it will help any of them, because it does not solve any of their real problems. The book contains 323 pages, is offered at $2.75 per copy, and is published by W. W. Norton & Co., New York.